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Determination of Plasma Fibrinogen Level in Diabetic Patients in Shendi Town

A thesis Submitted for Partial fulfillment of the Msc Degree in Medical Laboratory Sciences (Haematology)

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الآيـة

بسم الله الرحمن الرحيم

قال تعالى:

﴿ وَاخْفِضْ لَهُمَا جَنَاحَ الذُّلِّ مِنَ الرَّحْمَةِ وَقُل رَّبِّ ارْحَمْهُمَا كَمَا رَبَّيَانِي صَغِيراً ﴾

صدق الله العظيم

سورة الإسراء – الآية (24)

DEDICATION

Who To my parents …

encouraged me at all stages of life

To my brother and sisters …

For their unlimited support …

.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude and thankfulness to my supervisor

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For her guidance, meticulous supervision, revising and discussing all aspects of this study. Her valuable advices and comments are highly appreciated.

My great thanks also extend to the patients, others who contributed in a way or another for the success of this study especially.

List of abbreviation

Abbreviation	Term
ADP	Adinin Diposphat
Ag	Antigen
APTT	Activated Partial Thromboplastin Time
D.M	Diabetes Mellitus
DVT	Deep Vein Thrombosis
FDPs	Fibrin Degradation Products
GP	Glycoproteins
HMWK	High Molecular-Weight Kininogen
MW	Molecular Weight
PAI-1	Plasmin- Plasminogen Activator Inhibitor 1
PE	P ulmonary Embolism
PT	Prothrombin Time
TFPI	Tissue Factor Pathway Inhibitor
Tpa	Tissue-Plasminogen Activator
TT	Thrombin Time
VWF	Von Willebnllld Factor'

ABSTRACT

Background Diabetes mellitus is actually group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, action, or both. Thrombosis is one of the complications of diabetes; the coagulation system may be one of the affected systems.

Objective To evaluate the effect of Diabetes mellitus on plasma fibrinogen level in Shendi town

Methods: This is cross sectional study conducted in Shendi University in period between March and August 2018 to evaluate the effect of Diabetes mellitus on plasma fibrinogen level. Data collected, cleaned and analyzed using SPSS version (25.0).

Results: The study revealed that the mean of plasma fibrinogen among cases group was 389.8 mg/dl and among control group 244.2 mg/dl. The mean difference in the plasma fibrinogen level between the two study groups was 145.6%. This difference was statistically significant ($p < 0.001$). The study assessed the relation between numbers of factors on the level of plasma fibrinogen among the cases group. The study found a significant association with the level of plasma fibrinogen with the age of diabetic patients ($p = 0.0402$).

Conclusion and recommendation: Special attention should be given for patients who had the combination Diabetes and hyper-fibrinogenemia because that may increases the risk of developing micro and macro vascular complications. The control of diabetes should be assessed by further study to ensure if the elevation of fibrinogen level was significantly associated with uncontrolled diabetes in Sudan

الخلاصـة

الخلفية: مرض السكري هو مجموعة من الأمراض الأيضية التي تتميز بارتفاع السكر في الدم الناتج عن عيوب في إفراز الأنسولين، أو عمله، أو كليهما. تجلط الدم هو أحد مضاعفات مرض السكري. قد يكون نظام تجلط الدم أحد الأنظمة المتأثرة في الجسم وتؤدي إلى مضاعفات خطيرة.

الهدف: تقييم تأثير مرض السكري على مستوى الفيبرينوجين بالبلازما لسكان مدينة شندي المصابين بمرض السكري.

المنهجية: دراسة مقطعية أجريت بين المرضى بالسكري من سكان مدينة شندي بولاية نهر النيل في الفترة بين مارس وأغسطس 2018 لتقييم تأثير مرض السكري على مستوى الفيبرينوجين بالبلازما. البيانات تم جمعها وإعدادها وتحليلها باستخدامSPSS برنامج الإصدار 25.0.

النتائج: كشفت الدراسة أن متوسط الفيبرينوجين بالبلازما في جميع المشاركين بالدراسة كان 335.1 ملغ / ديسيلتر، من بين الحالات 389.8. مجم / ديسيلتر وبين مجموعة الكنترول 244.2. ملغ / ديسيلتر. بلغ متوسط الفرق في مستوى الفيبرينوجين في البلازما بين مجموعتي الدراسة 145.6٪. كان هذا الاختلاف مهمًا من الناحية الإحصائية .(p <0.001) قيمت الدراسة العلاقة بين عدد من العوامل واثرها على مستوى الفيبرينوجين بين مجموعة مرضى السكري. وجدت الدراسة ارتباطًا كبيرًا بمستوى الفيبرينوجين البلازمي مع عمر مريض السكري (P = 0.0402). .

الخاتمة والتوصيات: ينبغي إيلاء اهتمام خاص للمرضى الذين لديهم السكري وفرط في مستوى الفيزيرينوجين بالدم لأنه قد يزيد من خطر الإصابة بمضاعفات الأوعية الدموية الدقيقة والكلية. يجب تقييم السيطرة على مرض السكري من خلال مزيد من الدراسة لضمان ما إذا كان ارتفاع مستوى الفيبرينوجين مرتبطا بشكل كبير مع مرض السكري غير المنضبط في السودان.

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1.1 Introduction

Diabetes mellitus is actually group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, action, or both .[1] The National Diabetes Data Group developed a classification and diagnosis scheme for diabetes mellitus .this scheme dividing diabetes in to two broad categories; Type1 insuline, dependent diabetes mellites (IDDM) and type2 non insuline dependent diabetes mellites (NIDDM). [1]

The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycaemia sufficient to cause pathological diagnosis is made. The long–term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Several pathogenetic processes are involved in the development of diabetes [1]

1.2 Rationale

Diabetes mellitus is the most common non communicable diseases Characterized by hyperglycemia. It's widely distributed and effect on different organ. Thrombosis is one of the complications of diabetes; the coagulation system may be one of the affected systems. So this study was conducted to evaluate the effect of Diabetes mellitus on plasma fibrinogen level.

1.3 Objectives

1.3.1 General objective

Determination of plasma fibrinogen level in Diabetes mellitus.

1.3.2 Specific objectives

1. To measure fibrinogen level among diabetic patient and compared with control group.
2. To assess the effect of duration of disease on plasma fibrinogen level.
3. To correlate between types of diabetes and plasma fibrinogen level.
4. To determine the effect of gender in diabetes on plasma fibrinogen level.

Chapter Two

Literature review

2. Literature review

2.1: Haemostasis

Requires normal haemostatic response to vascular damage depends on closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors an efficient and rapid mechanism for stopping bleeding from sites of blood. The vessel injury is clearly essential for survival. Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The haemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process fibrinolysis. The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels [(2)]

2.2: Primary haemostasis

2.2.1 Platelets

2.2.1.1Platelet production

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte-the megakaryoblast-arises by a process of differentiation from the haemopoietic stem cell the megakaryocyte matures active, by end mitotic synchronous replication enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000-5000 platelets. The time interval from differentiation of the human stem cell to the production of platelets averages approximately 10 days. [(2)]

2.2.1.2: Platelet function

The main function of platelets is the formation of mechanical plugs during the normal hemostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. The immobilization of platelets at the sites of vascular injury specific platelet-vessel

wall (adhesion) and platelet-platelet (aggregation) interactions. The blood flow conditions determine the specific receptor ligand interactions. [2]

2.2.1.3: Platelet adhesion and activation

Following blood vessel injury, platelets adhere to exposed sub endothelial matrix proteins via specific adhesive glycoproteins (GP). Under condition high shear, e.g. arterioles, the exposed subendotrlial is it usually coated with VWF mealtime .The platelets than make contact with VWF via the GPIb-XI-V complex on platelets. This initiates platelet rolling in the direction of blood flow over the exposed VWF with activation of GPIIb/IlIa receptor. Firm adhesion is established by the slower stronger interaction of other glycoproteins inclutlg activated GPIIb/IlIa with VWF and GPVI and integrin (alpha1/beta 2) with collagen and other composer of the sub endothelial matrix. Under static or low shear conditions, platelets adhere predominantly to collagen of the sub endothelium. Collagen binds initially to GPIa/IIa, crosslinks many of these ingrain molecules, and in this way activates platelets This ligand receptor binding results in a complex cascade of signals which result in platelet activation The events that follow are shape change and spreading, activation of GPIIb/IIIa and granule section. Platelets become more spherical and extrude long pseudopodia which enhance platelet vessel wall and platelet-platelet interaction. The end result of spreading is a flattened spread out platelet with granules and organelles in the Centre, resulting in acharacteristic fried egg appearance. These changes are brought about by the actin cytoske. [2] Is involved in platelet adhesion to the vessel wall and to other platelets (aggregation). It also carries factor VIII and used to be referred to as factor VIII related antigen (VIII-Rag). It is a large cysteine-rich glycoprotein, with multimers made up on average of 2-50 subunits, with a molecular weight (MW) of 0.8-20 x 106. VWF is encoded by a gene on chromosome12 and is synthesized both in endothelial cells and megakaryocytes, and stored in Weiberl-Palade bodies and platelet alpha granules respectively. Plasma VWF is almost entirely derived from endothelial cells, with two distinct pathways of secretion. The majority is continuously

secreted and a minority is stored in Weibel-Palade bodies. The stored VWF can rise the plasma levels and it can be released under the influence of several secretagogues, like stress, exercise, adrenaline and infusion of decompressing (1-deamieno-8-D-arginine vasopressin, DDAVP). The VWF released from Seibel-Palade bodies is in the form of large and ultra large multiverse, the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to monomeric VWF and smaller multiverse by the specific plasma metalloprotease, ADAMTS-13.[2]

2.2.1.4: Platelet aggregation

It is characterized by cross-linking of platelets through active GPIIb/IIIa receptors with fibrinogen bridges. A resting platelet has about 50-80 000 GPIIb/IlIa receptors, which do not bind fibrinogen, VWF or other ligands. Stimulation of a platelet leads to an increase in GPIIb/Illa molecules, due to binding of alpha-granule membrane (rich in receptors) with the plasma membrane, activation of surface-exposed GPIIb/IlIa, enabling platelet cross-linking with fibrinogen bridges. Binding brings about molecular conformational changes resulting in a firm connection and further activation of the platelet.[2]

2.2.1.5: Clot formation and retraction

The highly localized enhancement of ongoing platelet activation by ADP and TXA2 results in a platelet plug large enough to plug the area of endothelial injury. In this platelet plug the platelets are completely DE granulated and adherent to each other. This is followed by clot retraction which is mediated by GPIIb/IlIa receptors which link the cytoplasmic actin filaments to the surface bound fibrin polymer. [2]

2.3: Secondary haemostasis

Secondary haemostasis involves a series of blood protein reactions through a cascade-like process that concludes with the formation of an insoluble fibrin clot. This system involves multiple enzymes and several cofactors as well as inhibitors to keep the system in balance. Coagulation factors are produced in the liver, except for factor VIII, which is believed to be produced in the endothelial

cells. When the factors are in a precursor form, the enzyme or zymogen is converted to an active enzyme or a protease. The initiation of clotting begins with the activation the initiation of clotting begins with the activation of two enzymatic pathways that will ultimately lead to fibrin formation: the intrinsic and extrinsic pathways. Both pathways are necessary for fibrin formation, but their activating factors are different. Intrinsic activation occurs by trauma within the vascular system, such as exposed endothelium. This system is slower and yet more important versus the extrinsic pathway, which is initiated by an external trauma, such as a clot and occurs quick.[3]

2.4: Classification of Coagulation Factors

Coagulation factors may be categorized into substrates, cofactors, and enzymes. Substrates are the substance upon which enzymes act. Fibrinogen is the main substrate. Cofactors accelerate the activities of the enzymes that are involved in the cascade. Cofactors include tissue factor, factor V, factor VIII, and Fitzgerald factor. All of the enzymes are serine proteases except factor XIII which is a transaminase.[4]

2.4.1: There are three groups in which coagulation factors can be classified

1. The fibrinogen group consists of factors I, V, VIII, and XIII. They are consumed during coagulation. Factors V and VIII are labile and will increase during pregnancy and inflammation.

2. The prothrombin group: Factors II, VII, IX, and X all are dependent on vitamin K during their synthesis. This group is stable and remains preserved in stored plasma.

3. The contact group: Factor XI, factor XII, prekallikrein, and high-molecular-weight kininogen (HMWK) are involved in the intrinsic pathway, moderately stable, and not consumed during coagulation.[4]

2.4.1.1: Factor I, Fibrinogen

Substrate for thrombin and precursor of fibrin, it is a large globulin protein. Its function is to be converted into an insoluble protein and then back to soluble components. When exposed to thrombin, two peptides split from the fibrinogen

molecule, leaving a fibrin monomer to form a polymerized clot. In general ,proteins are complex macro molecules composed of more than 40 amino acid linked by peptide bonds. Proteins are found in all cells and body fluid with the plasma alone containing more than 100 different species. The plasma proteins serve a large array of important function and exist in a variety of shape, size and structure. (5)

The one can separate the proteins of plasma into three major groups fibrinogen, albumin and globulin by the use of varying concentration of sodium and ammonium sulfate. (5)

Fibrinogen (factor I, 340 kD) is a soluble plasma glycoprotein that consists of three non -identical pairs of polypeptide chains, All three chains are synthesized in the liver; the three structural genes involved are on the same chromosome. (5)

Is large ,stable globulin protein ,it is precursor of fibrin with form the resulting clot. When fibrinogen is exposed to thrombin ,2peptide split from the fibrinogen molecule, leaving a fibrin monomers .these monomers aggregate together to form the final polymerized fibrin clot product**.** (6)

The fibrinogen gene cluster is located on chromosome 4q31 in the order γ α β with β transcribed in opposite direction to γ and α. the over all structure of fibrinogen is asymmetrical dimer α2 β2 ɣ2the molecule is trinodualar with the outer two globular domain containing the carboxytermini of all 3 chains .connected to central globular domain which contain the N-termini of all 6 chains tethered together by disulphide bonds, the lateral and central globular domains are connected by soiled coil regions forming α-helical ropes. Polymerization of fibrinogen occur when thrombin cleaves 2 short negatively charged fibrinopeptide A and B from the N-termini of the α and β chain respectively. (7)

It is classified as a glycoprotein because it has considerable carbohydrate content. On plasma electrophoresis, fibrinogen is seen as a distinct band between the β- and ɣ-globulins. The function of fibrinogen is to form a fibrin clot when activated by thrombin; therefore, fibrinogen is virtually all removed in

the clotting process and is not seen in serum. Fibrinogen customarily has been determined as clottable protein. Fibrinogen concentration is proportional to the time required to form a clot after the addition of thrombin to citrated plasma. Fibrin split products (degradation products of fibrinogen and fibrin) are determined by immunoassay methods such as radial immunodiffusion, nephelometry, and RIA.Fibrinogen is one of the acute-phase reactants, a term that refers to proteins that are significantly increased in plasma during the acute phase of the inflammatory process. Fibrinogen levels also rise with pregnancy and the use of oral contraceptives. Decreased values generally reflect extensive coagulation, during which the fibrinogen is consumed**.** [8]

2.4.1.2: Factor II, Prothrombin

Precursor to thrombin, in the presence of Ca2_, it is converted to thrombin (IIa), which in turn stimulates platelet aggregation and activates cofactors protein C and factor XIII. This is a vitamin K–dependent factor.

2.4.1.3: Factor III, Thromboplastin

Tissue factor activates factor VII when blood is exposed to tissue fluids.

2.4.1.4:Factor IV, Ionized Calcium:

This active form of calcium is needed for the activation of thromboplastin and for conversion of prothrombin to thrombin. **(3)**

2.4.1.5: Factor V, Proaccelerin or Labile Factor

This is consumed during clotting and accelerates the transformation of prothrombin to thrombin. A vitamin K dependent factor, 20% of factor V is found on platelets.

2.4.1.6:Factor VI, Nonexistent

2.4.1.7: Factor VII, Proconvertin or Stable Factor

This is activated by tissue thromboplastin, which in turn activates factor X. It is a vitamin K–dependent factor. **(3)**

2.4.1.8: Factor VIII, Ant hemophilic

This cofactor is used for the cleavage of factor X-Xa by IXa. Factor VIII is described as VIII/vWF:VIII:C active portion, measured by clotting, VIII:Ag is the antigenic portion, vWF:Ag measures antigen that binds to endothelium for platelet function; it is deficient in hemophilia A.

2.4.1.9:Factor IX, Plasma Thromboplastin Component

A component of the thromboplastin generating system, it influences amount as opposed to rate. It is deficient in hemophilia B, also known as Christmas disease. It is sex linked and vitamin K–dependent. [3]

2.4.1.10:Factor X, Stuart-Prowers

Final common pathway merges to form conversion of prothrombin to thrombin, activity also related to factors VII and IX. It is vitamin K–dependent and can be independently activated by Russell's viper venom. [3]

2.4.1.11:Factor XI, Plasma Thromboplastin Antecedent

Essential to intrinsic thromboplastin generating of the cascade, it has increased frequency in the Jewish population. Bleeding tendencies vary, but there is the risk of postoperative hemorrhage. [3]

2.4.1.12:Factor XII, Hageman factor

This surface contact factor is activated by collagen. Patients do not bleed but have a tendency to thrombosis. [3]

2.4.1.13:Factor XIII, Fibrin Stabilizing Factor

In the presence of calcium, this transaminase stabilizes polymerized fibrin monomers in the initial clot. This is the only factor that is not found in circulating plasma. [3]

2.4.1.14:High-Molecular-Weight Kininogen

This surface contact factor is activated by kallikrein. [3]

Prekallikrein, Fletcher Factor

This is a surface contact activator, in which 75% is bound to HMWK.[3]

2.5: Physiological Coagulation (In Vivo)

The original theory of coagulation used a cascade or waterfall theory. This description depicted the generation of thrombin by the soluble coagulation factors and the initiation of coagulation. This theory identified two starting points for the generation of thrombin: the initiation of the Intrinsic pathway with factor XII and surface contact, and the extrinsic pathway with factor VIIa and tissue factor. These two pathways meet at the common pathway, where they both generate factor Xa from X, leading to a common pathway of thrombin from prothrombin and the conversion of fibrinogen to fibrin. This process holds true under laboratory conditions The discovery of a naturally occurring inhibitor of hemostasis, tissue factor pathway inhibitor (TFPI), is able to block the activity of the tissue factor VIIa complex, soon after it becomes active9.[4]

2.6: Laboratory Model of Coagulation

Laboratory testing looks at the in vitro effect of the coagulation process which is measured by the prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrin degradation products (FDPs), and D-dimer. This section will focus on PT and a PTT. While the coagulation cascade does not reflect what goes on in vivo, it provides a model in which the laboratory relates to for testing. However, the coagulation cascade reflects the mechanisms that the laboratory uses for results. The screening tests provide a tremendous amount of information to the physician. They can be performed both quickly and accurately.[4]

2.7 Extrinsic Pathways

The extrinsic pathway is initiated by the release of tissue thromboplastin that has been expressed after damage to a vessel. Factor VII forms a complex with tissue thromboplastin and calcium. This complex converts factors X and Xa, which in turn converts prothrombin to thrombin. Thrombin then converts fibrinogen to fibrin. This process takes between 10 and 15 seconds.[3]

2.7.1:Prothrombin time

(PT) developed by Armand Quick in 1935 measures the extrinsic system of coagulation. It is dependent upon the addition of calcium chloride and tissue factor. It uses a lipoprotein extract from rabbit brain and lung. PT uses citrate anti coagulated plasma. After the addition of an optimum concentration of calcium and an excess of thromboplastin, clot detection is measured by an automated device for fibrin clot detection. The result is reported in seconds. PT is exclusive for factor VII, but this test is also sensitive to decreases in the common pathway factors. Therefore, if a patient presents with a prolonged PT and there is no other clinical abnormality or medication, the patient is most likely factor VII deficient. The PT is also used to monitor oral anticoagulation or warfarin therapy used to treat and prevent blood clots. In many instances, patients are placed on life-long therapy and the dosage is monitored by the PT test. The attempt in anticoagulant therapy is to impede thrombus formation without the threat of morbidity or mortality from hemorrhage. Warfarin is an oral anticoagulant, which means it must be ingested. It was discovered in 1939 at the University of Wisconsin quite by accident. It seems that a farmer found that his cattle were hemorrhaging to death, for what appeared to be no reason. The cattle were grazing in a field eating sweet clover. This contains dicumarol, actually bus hydroxyl Coumadin, which caused the cattle to bleed.[3]

2.7.2: There are several compounds of Coumadin

Dicumarol, indanedione, and warfarin. Dicumarol works too slowly, and indanedione has too many side effects. Warfarin or 4-oxycoumarin is the most commonly used oral anticoagulant. Coumadin works by inhibiting the y-carboxylation step of clotting and the vitamin K-dependent factors .[3]

2.8: Intrinsic System

Contact activation is initiated by changes induced by vascular trauma. Prekallikrein is required as a cofactor for the auto activation of factor XII by factor XIIa. XI is activated and requires a cofactor of HMWK. XIa activates IX to IXa, which in the presence of VIIIa converts X to Xa. Also present are

platelet phospholipids PF3. Calcium is required for the activation of X to proceed rapidly. The reaction then enters the common pathway where both systems involve factors I, II, V, and X. This results in a fibrin monomer polymerizing into a fibrin clot. Factor XIII, or fibrin stabilizing factor, follows activation by thrombin. This will convert initial weak hydrogen bonds, cross-linking fibrin polymers to a more stable covalent bond. (3)

2.8.1:Activated Partial Thromboplastin Time

APTT measures the intrinsic pathway. The test consists of decalcifying plasma in the presence of a standardized amount of platelet-like phosphatides and an activator of the contact factors. It will detect abnormalities to factors VIII, IX, XI, and XII. The APTT is also used to monitor heparin therapy. Heparin is an anticoagulant used to treat and or prevent acute thrombotic events such as deep vein thrombosis (DVT), pulmonary embolism (PE), or acute coronary syndromes. The action of heparin is to inactivate factors XII, XI, and IX in the presence of anti-thrombin. (3)

2.9: Common Pathways

The common pathway is the point at which the intrinsic and extrinsic pathways come together and factors I, II, V, and X are measured. It is important to note that the PT and the APTT will not detect qualitative or quantitative platelet disorders, or a factor XIII deficiency. Factor XIII is fibrin stabilizing factor and is responsible for stabilizing a soluble fibrin monomer into an insoluble fibrin clot. If a patient is factor XIII deficient, the patient will form a clot but will not be able to stabilize the clot and bleeding will occur later. Factor XIII is measured by a 5 mol/L urea test that looks at not only the formation of the clot but also if the clot lazes after 24 hours. (3)

Formation of Thrombin

When plasma fibrinogen is activated by thrombin, this conversion results in a stable fibrin clot. This clot is a visible result that the action of the protease enzyme thrombin has achieved fibrin formation. Thrombin is also involved in the XIII-XIIIa activation due to the reaction of thrombin cleaving a peptide bond

from each of two alpha chains. Inactive XIII along with Ca2_ ions enables XIII to dissociate to XIIIa. If thrombin were allowed to circulate in its active form (Ia), uncontrollable clotting would occur. As a result thrombin circulates in its inactive form prothrombin (II).Thrombin, a protease enzyme, cleaves fibrinogen (factor I) which results in a fibrin monomer and fibrinogen peptides A and B. These initial monomers polymerize end to end due to hydrogen bonding.

Formation of fibrin occurs in three phases:

1. Proteolysis: Protease enzyme thrombin cleaves fibrinogen resulting in a fibrin monomer, A and B fibrin peptide.
2. Polymerization: This occurs spontaneously due to fibrin monomer that line up end-to-end due to hydrogen bonding.
3. Stabilization: This occurs when the fibrin monomers are linked covalently by XIIIa into fibrin polymers forming an insoluble fibrin clot. [3]

Feedback Inhibition

Some activated factors have the ability to destroy other factors in the cascade. Thrombin has the ability to temporarily activate V and VIII, but as thrombin increases it destroys V and VIII by proteolysis. Likewise, factor Xa enhances factor VII, but through a reaction with tissue factor pathway inhibitor (TFPI), it will prevent further activation of X by VIIa and tissue factor. Therefore, these enzymes limit their own ability to activate the coagulation cascade at different intervals. Thrombin feedback activation of factor IX can possibly explain how intrinsic coagulation might occur in the absence of contact factors. Tissue factor is expressed following an injury forming a complex with VIIa, then activating X and IX. TFPI prevents further activation of X. Thrombin formation is further amplified by factors V, VIII, and XI, which leads to activation of the intrinsic pathway. This feedback theory helps to enforce why patients with contact factor abnormalities (factors XI and XII) do not bleed. [3]

2.10: Fibrinolysis

The fibrinolysis system is responsible for the dissolution of a clot. Fibrin clots are not intended to be permanent. The purpose of the clot is to stop the flow of blood until the damaged vessel can be repaired. The presence or absence of hemorrhage or thrombosis depends on a balance between the procoagulant and the fibrinolysis system. The key components of the system are plasminogen, plasminogen activators, plasmin, fibrin, fibrin/ FDP, and inhibitors of plasminogen activators and plasmin.6 Fibrinolysis is the process by which the hydrolytic enzyme plasmin digests fibrin and fibrinogen, resulting in progressively reduced clots. This system is activated in response to the initiation of the activation of the contact factors. Plasmin is capable of digesting either fibrin or fibrinogen as well as other factors in the cascade (V, VIII, IX, and XI). Normal plasma contains the inactive form of plasmin in a precursor called plasminogen. This precursor remains dormant until it is activated by proteolysis enzymes, the kinases, or plasminogen activators. Fibrinolysis is controlled by the plasminogen activator system. The components of this system are found in tissues, urine, plasma, lysosome Granules, and vascular endothelium. An activator, tissue-plasminogen activator (tPA) results in the activation of plasminogen to plasmin resulting in the degradation of fibrin. The fibrinolysis system includes several inhibitors. Alpha-2-antiplasmin is a rapid inhibitor of plasmin activity and alpha- 2 macroglobulin is an effective slow inhibitor of plasmin activity. This system is in turn controlled by inhibitors to tPA and plasmin-plasminogen activator inhibitor 1 (PAI-1) and alpha-2-antiplasmin. Reduced fibrinolytic activity may result in increased risk for cardiovascular events and thrombosis. Pharmacologic activators are currently used for therapeutic thrombolysis, including streptokinase, urokinase, and tPA. Urokinase directly activates plasminogen into plasmin, and streptokinase forms a streptokinase plasminogen complex, which then converts plasminogen into plasmin.[(2)]

2.11: Coagulation Inhibitors

Inhibitors are soluble plasma proteins that are natural anticoagulants. They prevent the initiation of the clotting cascade. There are two major inhibitors in plasma that keep the activation of coagulation under control**.** [9]

These inhibitors are:

1. Protease inhibitors: inhibitors of coagulation factors, which include

• Antithrombin.

• Heparin cofactor II.

• Tissue factor pathway inhibitor.

• Alpha-2-antiplasmin.

• C1.

2. The protein C pathway: inactivation of activated cofactors, which includes

• Protein C and protein S.

Kinin System

Another plasma protein system in coagulation is the kinin system. This system is capable of vascular dilatation leading to hypotension, shock, and end-organ damage by its capability to increase vascular permeability.

The kinins are peptides of 9 to 11 amino acids. The kinin system is activated by factor XII. Hageman factor XIIa converts prekallikrein (Fletcher factor) into kallikrein, and kallikrein converts kininogens into kinins. The most important is bradykinin (BK). This is an important factor in vascular permeability as well as a chemical mediator of pain. BK is capable of reproducing many characteristics of an inflammatory state such as changes in blood pressure, edema, and pain, resulting in vasodilation and increased microvessel permeability. [3]

Complement System

This system has a role in inflammation and the immune system as well as important thrombohemorrhagic disorders such as disseminated intravascular coagulation (DIC). Activated complement fragments have the capacity to bind and damage self-tissues. Regulators of complement activation are expressed on cell surfaces. These protect the cell from the effects of cell-bound complement

fragments. If this regulation process is abnormal, it may participate in the pathogenesis of autoimmune disease as well as inflammatory disorders. This system includes 22 serum proteins that play a role in mediating immune and allergic reactions and the lysis of cells due to a production of membrane attack complex (MAC). The lysis and disruption of red blood cells and platelets lead to the release of procoagulant material. This system is a sequential activation pathway. Complement is activated by plasmin through the cleavage of C3 into C3a and C3b. C3 causes increased vascular permeability, and because of the degranulation or lysis of mast cells, which in turn results in the release of histamine, C3b causes immune adherence. The interrelationship between the complement, kinin, and the coagulation system is complex and revealing. Coagulation and the elements that contribute to the success of the hemostatic system are multifactorial, and with each decade, more knowledge about this versatile system is learned. Illustrates the important interrelationships between the coagulation, fibrinolytic, complement, and kinin systems. [3]

2.12: Diabetes mellitus (D.M)

Diabetes mellitus is actually of metabolic disease characterized by hyperglycemia resulting from defect in insulin secretion insulin action or both.[10]

- Diabetes mellitus is caused by an absolute or relative insulin deficiency .it has be defined by the world health organization (WHO)on basis on the laboratory findings as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140mg/dl).or greater than 11.1 mmol/l (200mg/dl) 2hour after carbohydrate meal or 2hour after the oral ingestion even if the fasting concentration normal .[10]

The term diabetes, without qualification, usually refers to diabetes mellitus, which roughly translates to excessive sweet urine (known as "glycosuria. Several rare conditions are also named diabetes. The most common of diabetes insipidus in which large amounts of urine are produced hose is (polyuria, which is not sweet (insipid us meaning "without taste" in Latin). [11]

2.13: Classification of diabetes mellitus

Diabetes mellitus is classified into the following categories:

(1)-Insulin dependent diabetes mellitus (IDDM- type I):

Is term use to describe the condition in patient for whom insulin therapy.[12] Is essential they are prone to develop ketoacidosis, is characterize by deficient insulin production and daily administration of insulin. Type (1) diabetes mellitus is result of cellular mediate auto immune destruction of the B cell of the pancrease, causing an absolute deficiency of insulin secretion. Type (1) constitute only 10% to 20% of all cases of diabetes and commonly occur in child hood and adolescence.[1]

(2)- Non insulin dependent diabetes mellitus (NIDDM- type 2):

Patients are much less likely to develop ketoacidosis than those with IDDM and although insulin may some times be needed [12]. Type (2) diabetes is characterized by hyperglycemia as result of an indivual's resistance to insulin with an insulin secretory defect, this resistance result in arelative, not an absolute, insulin deficiency. Type (2) diabetes comprise 90% of people with diabetes around the world.[1]

(3)-Diabetes mellitus associated with other condition

Includeabsolute insulin deficiency: due to excessive growth hormone (acromegal Glycocorticoid secretion cushing syndrome or increased administration of steroids. Drug such as thiazid diureticus .[12]

(4)-Gestational diabetes mellitus:

Any degree of glucose into lerance with onset or first recognition during pregnancy. Causes of GDM include frequent reteun to normal post partum. Hower this disease associated with increase risk for development complication and increase risk for development of diabetes in later years.[1]

Infant born to mother with diabetes are increase risk for respiratory distress syndrome, hypocalcemia and hyper bilirubinemia. Impaired glucose to lerance and impaired fasting glycemia (IGI).

Impaired glucose to lerance and impaired fasting glycemia (IFG) are

intermediate condition in the transition between normality and diabetes. People with IGT and IFG are at high risk for progressing of type 2 diaetes. [13]

2.14: Differences between type 1&2 diabetes

• Approximately 10% of diabetics are of the type 1 variety.

• The type 1 disease state usually occurs as acute illness, while type 2 diabetes progresses slowly over time.

• Type 1 glucose blood levels are usually more severe than type 2.

• Type 1 diabetics are more likely to develop ketoacidosis than are type 2 diabetics. Due to the etiology of disease.

• type 1 diabetics are insulin dependent, while most type 2 diabetics are not.

• Type 1 diabetics are younger (18 years old when diagnosed) and thinner; type 2 diabetics are usually older (40 years old when diagnosed) and more likely to be obese.

• However, these characteristics of presentation are not uniform to all type 1 and type 2 diabetics. Type 1 diabetes may be diagnosed after the age of 18 years. Type 2 diabetes may develop in obese children. Type 2.diabetics may need insulin if glycaemia cannot be controlled by other measures**.** [11]

2.15: Signs and symptoms

Overview of the most significant symptoms of diabetes. The classical symptoms of diabetes are polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Symptoms may develop rapidly (weeks or months) in type 1 diabetes while in type 2 diabetes they usually develop much more slowly and may be subtle or absent. Prolonged high blood glucose causes glucose absorption, which leads to changes in the shape of the lenses of the eyes, resulting in vision changes; sustained sensible glucose control usually returns the lens to its original shape. Blurred vision is a common complaint leading to a diabetes diagnosis; type 1 should always be suspected in cases of rapid vision change, whereas with type 2 changes is generally more gradual, but should still be suspected. People (usually with type 1 diabetes) may also present with diabetic ketoacidosis, a state of metabolic deregulation characterized by the

smell of acetone; a rapid, deep breathing known as Kussmaul breathing; nausea; vomiting and abdominal pain; and an altered states of consciousness. A rarer but equally severe possibility is hyperosmolar non ketosis state, which is more common in type 2 diabetes and is mainly the result of dehydration. Often, the patient has been drinking extreme amounts of sugar-containing drinks, leading to a vicious circle in regard to the water loss. A number of skin rashes can occur in diabetes that are collectively known as diabetic dermadromes. (13)

2.16*:* Complications of Diabetes

What are the common consequences of diabetes:

Over time, diabetes can damge the heart, blood vsessels, eyes, kidney and nerves. Diabetes increase the risk of heart disease and stroke. (1)

50% of people with diabetes die of cardiovascular disease (primarily heart disease and stroke). Combined with reduce blood flow, neuropathy in the feet increase the chance of foot ulcer and eventual limb amputation .(1)

Diabetic retinopathy an important causes of blindness, and occur as result of long term accumulated damgae to small blood vessels in the retina. After 15years of diabetes approximately 2% of people become blind, and about 10% develop sever visual impairment. Diabetes is among the leading causing of kidney failure. 10 – 20% of people with diabetes die of kidney failure. Diabetic neuropathy is damage to the nerves as result of diabetes, and affect up to 50% of people with diabetes. Although many different problem can occur as result of diabetic ,neuropathy or weakness in the feet and hands. (13)

2.16.1: Metabolic complications of diabetes

2.16.1.1:Ketoacidosis:

May the presenting feature of type 1 DM, or may develop in a patient known to be diabetic who omits to take his insulin or whose insulin dosage becomes inadequate because of an increased requirement. Clinical and metabolic features of diabetic ketoacidosis:

Clinical: thirst, polyuria, dehydration, hypotension, tachycardia, vomiting hyperventilation, abdominal pain, coma. Metabolic: hyperglycaemia, glycosuria,

non-respiratory acidosis, ketonaemia and ketonuria, uraemia, hyper kalaemia, haemoconcentration, hypertriglyceridaemia. [13]

2.16.1.2:Non-ketotic hyperglycaemia

In type 2 DM, severe hyperglycaemia can develop (blood glucose concentration >50 mmol/l) with extreme dehydration and a very high plasma osmolality ,but with no ketosis and minimal acidosis. [13]

2.16.1.3:Lactic acidosis

Is an uncommon complication of diabetes. It was formely chiefly seen in patients treated with phenformin, oral hypoglycaemic drug, but is now more usually associated with severe systemic illness, e.g.: shock and pancreatitis. [13]

2,16.2: Diabetic nephropathy

Is a major cause of prematyre death in patient with diabetes, related with cardiovascular disease as well as renal failure .the earliest detectable abnormality is microalbuminuria, proteinuria, increasing plasma creatinine, hyperlipidemia and hypertension. [13]

2.16.3: Hypoglycemia

Defined as a blood glucose concentration of less than 2.8mmol/l, hypoglycaemia is a potentially dangerous. [13]

2.16.3.1: Causes

1. Fasting hypoglycaemia (hepatic and renal disease, endocrine disease, metabolic disorder, hyperinsulinism, alcoholic induced, septicaemia).
2. Reactive hypoglycaemia (post-prandial, drug induced, inherited metabolic disorder)
3. Clinical features.
4. Acute: Neuroglycopenia (tiredness, confusion, ataxia, dizziness, coma, sympathetic stimulation (palpitation, tachycardia, sweating, tremor) and non-spesific (weakness, hunger, blurred vision).
5. Chronic: Neuroglycopenia (memory loss, psychosis, dementia. [13]

2.17 Previous studies

2.17.1 A study by S. M. Alnour, et al. in India 2012 assessed Fibrinogen Level in Type 1 and Type 2 Diabetes Mellitus.

The study included 60 diabetic patients (30 type 1 DM and 30 type 2 DM patients) their fibrinogen level, were measured and compared with 30 normal subjects as control. Fibrinogen level was measured by Clauss modified method. This study revealed higher fibrinogen level among diabetic patients than the control, with further increase in the fibrinogen level among type 2 DM patients than type 1 patients. This study also demonstrated significant correlation between fibrinogen level and HbA1C level. In conclusion, this study revealed higher fibrinogen level among diabetic patients than the control, this finding suggested an increased cardiovascular risk among DM patients; the risk is increased with poor glycemic control. [15]

2.17.2 Archana etal conducted a Study of Plasma Fibrinogen Level in Type-2 Diabetes Mellitus in India 2012.

This study is undertaken to know the fibrinogen levels in type 2 diabetes mellitus and its relations to glycemic control. In the present study fibrinogen levels (Clauss method) were estimated in 100 type 2 diabetic subjects and 100 age and sex matched controls. Fibrinogen was correlated with various parameters like glycosylated hemoglobin (cation exchange resin method), age, sex, smoking, body mass index (kg/m2), hypertension and ischemic heart disease. Higher plasma fibrinogen levels were found in type 2 diabetes mellitus patients (656 ± 130 mg/dl) as compared to controls (324 ± 139 mg/dl) which were statistically significant. Fibrinogen levels were associated with age ($P < 0.01$), hypertension ($P < 0.01$), body mass index ($P < 0.01$), smoking ($P < 0.01$), ischemic heart disease ($P < 0.01$), and glycosylated hemoglobin ($r = 0.49$) in diabetics in a significant manner. But no correlation was found with sex ($P < 0.05$) in diabetes. In controls, association was found between fibrinogen levels and smoking ($P < 0.01$) and body mass index ($P < 0.01$). Patients with type 2 diabetes mellitus had a high prevalence of hyperfibrinogenemia. Fibrinogen

levels were independently associated with hemoglobin A1c values, which suggests that fibrinogen may be involved in the increased cardiovascular risk of patients with type 2 diabetes mellitus. [16]

2.17.3 Shihabi et al measured Plasma fibrinogen levels in type II diabetics in US 2008.

In this study, plasma fibrinogen levels measured by an immunoassay method on 170 type II diabetic patients exhibited a bimodal distribution with one small population demonstrating levels greater than those of the normal reference range. The mean plasma level of fibrinogen in the type II diabetics was higher than that of the normal population. Spearman's correlations demonstrated statistically significant positive relationships in type II diabetic patients between fibrinogen levels and fasting glucose levels, serum cholesterol, glycosylated hemoglobin and urinary albumin excretion rate. These relationships suggest that increased plasma fibrinogen may be another marker for coronary heart disease complications encountered by diabetics. [17]

2.17.4 Other study conducted by Enass A. M. Khalid etal in Sudan 2012 that measured fibrinogen level among type 2 diabetic Sudanese patients.

Fibrinogen level was measured by Clauss modified method. Fibrinogen level was found to be significantly higher in diabetic patients, with no difference between males and females. Fibrinogen levels were significantly associated with HbA1c levels (p value 0.000) and the duration of diabetes (p value 0.048). The study concluded that fibrinogen level is higher among type 2 diabetic patients. The elevation of fibrinogen level was significantly associated with uncontrolled diabetes. [18]

2.17.5 Other study by Bembde AS et al. in India 2012 investigated the level of plasma fibrinogen level in type-2 diabetes mellitus.

In the present study fibrinogen levels (Clauss method) were estimated in 100 type 2 diabetic subjects and 100 age and sex matched controls. Fibrinogen was correlated with various parameters like glycosylated hemoglobin (cation exchange resin method), age, sex, smoking, body mass index (kg/m(2)),

hypertension and ischemic heart disease. Higher plasma fibrinogen levels were found in type 2 diabetes mellitus patients (656 ± 130 mg/dl) as compared to controls (324 ± 139 mg/dl) which were statistically significant. Fibrinogen levels were associated with age ($P < 0.01$), hypertension ($P < 0.01$), body mass index ($P < 0.01$), smoking ($P < 0.01$), ischemic heart disease ($P < 0.01$), and glycosylated hemoglobin ($r = 0.49$) in diabetics in a significant manner. But no correlation was found with sex ($P < 0.05$) in diabetes. In controls, association was found between fibrinogen levels and smoking ($P < 0.01$) and body mass index ($P < 0.01$). Patients with type 2 diabetes mellitus had a high prevalence of hyperfibrinogenemia. Fibrinogen levels were independently associated with hemoglobin A1c values, which suggests that fibrinogen may be involved in the increased cardiovascular risk of patients with type 2 diabetes mellitus. [19]

2.17.6 Other study conducted by R.S. Nagaraj etal. In JCDR 2015 Addressed Plasma Fibrinogen in Type 2 Diabetic Patients.

The study found that fifty eight patients have hyperfibrinogenemia and mean fibrinogen level is significantly high in diabetic patients when compared to non-diabetic patients ($p<0.001$). Diabetic patient. The study concluded that the combination Diabetes and hyperfibrinogenemia increases the risk of developing micro and macro vascular complications. [20]

2.17.7 R. Barazzoni etal investigated the increased Fibrinogen Production in Type 2 Diabetic Patients J clin Endocrinol metab. 2000.

The study found that diabetic patients also had increased plasma fibrinogen concentration (+50%; $P < 0.01$) and pool (+40%; $P < 0.01$) as well as fractional (+35%; $P = 0.08$) and absolute (+100%; $P < 0.01$) synthetic rates. The plasma glucagon concentration was positively related ($P < 0.005$ or less) to the fibrinogen concentration as well as to fractional and absolute synthetic rates. Thus, fibrinogen production is markedly enhanced, and this alteration is likely to determine the observed hyperfibrinogenemia in type 2 diabetic patients. Hyperglucagonemia may contribute to the increased fibrinogen production. These findings in normoalbuminuric patients without clinical complications

support the hypothesis that increased fibrinogen production and plasma concentrations may precede and possibly contribute to the onset of clinical cardiovascular complications in type 2 diabetes. [21]

Chapter Three

Materials and Method

3 Materials and Method

3.1 Study design

Across sectional descriptive study, conducted in Shendi town in the period from March to August 2018 that aimed to measure the fibrinogen level in patients with diabetes mellitus.

3.2 Study area

The study was done in Shendi town which is located in the north of Sudan and north of the capital Khartoum and for about 173km, and to the south of Aldamer for about 127km, located in the east side of the river Nile, and covering area about 30km, the population about .most of people are farming. It contain three hospitals and health centers, also there is Shendi University with various faculties like faculty of medicine and health science.

3.3 Study population

Eighty samples collected form study groups from (50) diabetic patient and 30 controls

3.4 Sample

Venous blood collected using sterile disposable plastic syringe after cleaning the venepuncture area with 70% ethanol, the blood was add to the anticoagulant at ratio of 2.5ml to0.5ml of citrate (3.2% (0.109M) buffered sodium citrate and gently mixed. The sample was centrifuge at 1300 rpm for 15min to obtain platelet poor plasma (ppp). The ppp placed into plastic tube

3.5 Data collection tools

The primary data was collected by using questionnaire.

3.6 Data analysis

Data was entered, cleaned, and analyzed using SPSS version 25.0 using T-test to p.vlue.

3.7 Method

The TE Clot is intended for the quantitative determination of fibrinogen in human plasma according to method developed by Clauss.

3.7.1.principle of Clauss

Diluted plasma is clotted with a strong thrombin solution the plasma most be diluted to give a low level of any inhibitors (eg : FDPs and heparin). A strong thrombin solution must be used so that the clotting time over a wide range is independent ot thrombin concentration

3.7.2 Contents

1. Thrombin Reagent (Bovine Thrombin)
2. IBS Buffer
3. TE Control Normal
4. TE control A

3.7.3 Specimen collection and storage

1. Obtain venous blood by clean vein puncture
2. Immediately mix 9 parts blood with 1 part 3.2% sodium citrate (0.105M) and mix well
3. Centrifuge the specimen at 1500g for 10min
4. Separate plasma after centrifugation and store in plastic tube
5. Use plasma within 4fours. Otherwise store frozen and thaw just prior to use

3.7.4. procedures

3.7.4.1 Automated Method. Coatron A

3.7.4.2 Manual Method (Coatron) M

3.7.4.3 Preparation of standard, Control and patient dilutions

Standard Dilution	Plasma	IBS Buffer
1:5	200µl Standard	800µl
1:10	500µl 1:5 STD	500µl
1:20	500µl 1:10 STD	500µl
1:40	500µl 1:20 STD	500µl
Patient or control	100µl Plasma	900µl

1.10.2.2 Pipette 50µl diluted standard or patient plasma ;(1:10) into test cuvette prewarm at 37ºC for 1-2 minutes1.10.2.3 Add 25µl thrombin reagent and simultaneously start test.

For other instrument. Please refer to your instrument manual for more detailed instrument specific instructions

Normal Range: 180-450mg/dl

3 .8 Ethical consideration

An informed consent from selected individuals was obtained after being informed with all detail objective of the study.

Chapter Four

Results

4. Results

This study covered 80 study participants, divided into two study groups. Fifty participants who diagnosed with diabetes/cases group (62%) and 30 healthy participants (38%) acted as control group as showed in table 1.

Concerning the age, about two thirds of the study participants were more than 40 years in age (78%) whiles only (22%) of them were less than 40 years in age. The age distribution was almost similar between the two study groups as detailed in table 2.

Similarly, more than half of the study participant (56%) were males. Likewise, the gender distribution was not differ in both study groups as illustrated in table2.

More than three quarters (78%) were from diabetes type 2 while only (22%) with type I. Furthermore, nearly third of cases were diabetic for more than 5 years (32%) and less than or equal 5 years (68%) as detailed in tables 3 - 4 below.

Regarding the presence of other disease among cases group, the study found that the most prevalent disease among cases group were hypertension (28%), Thrombosis (6%), as detailed in table 5 .

The study found that nearly three quarters of cases group (72%) had regular treatment, while (26%) were irregular in taking their treatment as showed in table 6 below.

The study revealed that the overall plasma fibrinogen in all study participates was 335.1 mg/dl, among cases group 389.8 mg/dl and among control group 244.2 . mg/dl. The mean difference in the plasma fibrinogen level between the two study groups was 145.6%. This difference was statistically significant ($p < 0.001$).

The study assessed the relation between numbers of factors on the level of plasma fibrinogen among the cases group. The study found a significant association with the level of plasma fibrinogen with the age of diabetic patients ($p = 0.0402$).

Table (4-1): Distribution of the study group

Study groups	Frequency	Percent
Cases	50	62%
Controls	30	38%
Total	80	100%

Table (4-2): Distribution of the study group according to the age and gender

General characteristics		Study groups			
		Cases (n = 50)		Controls (n = 30)	
		Frequency	Percent	Frequency	Percent
Age – years	≤ 40	11	22%	15	20%
	> 40	39	78%	15	50%
Gender	Male	28	56.%	16	53%
	Female	22	44%	14	47%

Table (4-3): Distribution of the study group according to the type of diabetes

Type of diabetes	Frequency	Percent
Type I	11	22%
Type II	39	78%
Total	50	100%

Table (4-4): Distribution of the study group according to the duration of the disease

Duration of the disease - years	Frequency	Percent
≤ 5	34	68%
> 5	16	32%
Total	50	100%

Table (4-5): Distribution of the study group according to risk Factor

Other diseases	Frequency	Percent
Hypertension	14	28%
Thrombosis	3	6%
None	33	66%
Total	50	100%

Table (4-6): Distribution of the study group according to the treatment regularity

Treatment regularity	Frequency	Percent
Regular	36	72%
Irregular	14	28%
Total	50	100%

Table (4-7):Mean of plasma fibrinogen level in cases and control

Study group	Mean	P. value
Cases	389.8	< 0.001
Controls	244.2	

Table (4-8): Mean of plasma fibrinogen according to age.

Age – years	Mean	P value
≤ 40	361.3	0.0402
> 40	402.3	

Table (4-9): Mean of plasma fibrinogen according to gender

Gender	Mean	P value
Male	325.6	0.3280
Female	343.1	

Table (4-10): Mean of plasma fibrinogen according to DM type

DM type	Mean	P. value
Type I	389.0	0.0625
Type II	392.5	

Table (4-11): Mean of plasma fibrinogen according to DM duration

DM duration – years	Mean	P. value
≤ 5	384.9	0.7471
> 5	400.7	

Table (4-12): Mean of plasma fibrinogen according to Treatment regularity

Treatment regularity	Mean	P. value
Regular	376.4	0.9353
Irregular	387.5	

Table (4-13): Mean of plasma fibrinogen according to Other diseases

Other diseases	Mean plasma fibrinogen
Hypertension	460.3
Thrombosis	411.7

Chapter Five

Discussion

Conclusion

Recommendations

5.1 Discussion

This study aimed to measure the level of plasma fibrinogen among diabetic patients in Shendi Town during the period from March to Augusts 2018. This study include 80 study participants, divided into two groups, Fifty participants who diagnosed with diabetes (cases) (62.5%) and 30 healthy participants (37.5%) acted as control group.

Concerning the age, about two thirds of the study participants were more than 40 years in age (67.5%) whiles only (21.3%) of them were less than 40 years in age. The age distribution was almost similar between the two study groups. In a study from India they found no correlation was found with sex ($p = 0.154$), hypertension ($p = 0.167$), duration of diabetes ($p = 0.06$), and smoking ($p = 0.283$) in cases. In controls, plasma fibrinogen level was associated with age ($p = 0.004$) and body mass index ($p = 0.0008$). [15]

More than three quarters (78%) were from diabetes type 2 while only (22%) with type I. Furthermore, nearly third of cases were diabetic for more than 5 years (32%).

Regarding the presence of other disease among cases group, the study found that the most prevalent disease among cases group were hypertension (28%), Thrombosis (6%), [16]

The study revealed that the overall plasma fibrinogen in all study participates was 335.1 mg/dl, among cases group 389.8 mg/dl and among control group 244.2 mg/dl. The mean difference in the plasma fibrinogen level between the two study groups was 145.6%. This difference was statistically significant ($p < 0.001$). These results were in agreement with other study by Archana etal. They found a Higher plasma fibrinogen levels were found in type 2 diabetes mellitus patients (656 mg/dl) as compared to controls (324 ± 139 mg/dl) which were statistically significant. p.value <0.01 [16] other study conducted The mean

plasma level of fibrinogen in the type II diabetics was higher than that of the normal population.

The results of our study was in agreement with other American study by Shihabi et al. who found that the mean plasma level of fibrinogen in the type II diabetics was higher than that of the normal population. [17]

The study assessed the relation between numbers of factors on the level of plasma fibrinogen among the cases group. The study found a significant association with the level of plasma fibrinogen with the age of diabetic patients ($p = 0.0402$). Other study conducted in India by Archana etal showed that fibrinogen levels were associated with age ($P < 0.01$. In the same context, other study by R. Barazzoni etal investigated the increased Fibrinogen Production in Type 2 Diabetic Patients. The study found that diabetic patients also had increased plasma fibrinogen concentration (+50%; $P < 0.01$) and pool (+40%; $P < 0.01$) as well as fractional (+35%; $P = 0.08$) and absolute (+100%; $P < 0.01$) synthetic rates.

The study did not found a significant relationship between the level of plasma fibrinogen among diabetes with the type of diabetes ($p = 0.0625$). this results was differ from the study done in Sudan by S. M. Alnour, et al. who found that a higher fibrinogen level among diabetic patients than the control, with further increase in the fibrinogen level among type 2 DM patients than type 1 patients.[15]

The study found no significant difference in the level of plasma fibrinogen among diabetic patients according to the gender($p = 0.328$). This result was similar to other study conducted in Sudan by Enass A. M. Khalid et al they found that Fibrinogen level was found to be significantly higher in diabetic patients, with no difference between males and females, p.value 0.048. [18]

5-2 Conclusion

1-Mean of plasma fibrinogen level in patient with diabetics mellitus were 389.8.mg/dl while in control were244,2mg/dl.

2-Mean of plasma fibrinogen level according the age less than 40 were361.3 mg/dl while in more than 40 were402.3mg/dl.

3-The mean difference in the plasma fibrinogen level between the tow study groups was 145.6%. This difference was statistically significant ($p<0.001$).

4- patients with diabetic mellitus has significant increased in fibrinogen level, which may indicator hypercoaguable factor that may lead to thrombotic tendency.

5.3 Recommendation

1- Special attention should be given for patients who had the combination Diabetes and hyper-fibrinogenemia because that may increases the risk of developing micro and macro vascular complications.

2- The control of diabetes should be assessed by further study to ensure if the elevation of fibrinogen level was significantly associated with uncontrolled diabetes in Sudan

3- Further studies were recommended to assess the possible relationships between increased plasma fibrinogen may be another marker for coronary heart disease complications encountered by diabetics among Sudanese patients.

4- Based on our findings, we suggest increased plasma fibrinogen can be prevented by promotion of glycemic control

Chapter Six

References

Appendices

6.1 References

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Appendix

Questioner

SHENDI UNIVERSITY

FACULITY OF GRADUATE STUDIES & SCI ENTIFIC RESEARCH

Research questioner:

Effect of Diabetes mellitus on plasmafibrinogen level

1-Name:……..……NO ( )

2-Age: less than 40Years ( ) more than 40 year ( )

3-Gender: male ( ) female ( )

4-Type of diabetes:

Type I ( ) Type II ( )

5-Duration of disease:

Less than 5years ( ) more than 5 years ( )

6- Are you complain from any of these disease?

Renal disease ( ), liver disease ( ), Hypertension , , thrombosis ( )

7-Treatmet: regular ( ), irregular ( ),

……

Special for researcher:

Plasma fibrinogen level: (…… mg/dl)