Napata College
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Determination of prothrombin time and activated partial thromboplastin time among diabetic patients attending in Military Hospital in Khartoum state

A Dissertation submitted in partial fulfillment of the requirement of B.SC. in Medical Laboratory Sciences (Hematology)

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الآية

بسم الله الرحمن الرحيم

قال الله تعالي:

(ثُمَّ كُلِي مِنْ كُلِّ الثَّمَرَاتِ فَاسْلُكِي سُبُلَ رَبِّكِ ذُلُلاً ۚ يَخْرُجُ مِنْ بُطُونِهَا شَرَابٌ مُخْتَلِفٌ أَلْوَانُهُ فِيهِ شِفَاءٌ لِلنَّاسِ ۗ إِنَّ فِي ذَٰلِكَ لَآيَةً لِقَوْمٍ يَتَفَكَّرُونَ)

صدق الله العظيم

سورة النحل الآية رقم(٦٩)

Dedication

We dedicate our work to our families and many friends and colleagues, A special feeling of gratitude to our loving parents whose words of encouragement and push for tenacity ring in our ears.

Acknowledgment

we would like to give a heartfelt thanks to our be loving and amazing mentor and teacher

Mrs. Sally Magdi

Thank you for your kindness, patience and continues support and inspirational instruction and guidance thank you for making this a tremendous experience for us. She consistently makes herself available to her students, to listen and encourage them to pursue their desired interests. she taught us how to break through the obstacles to get things done.

we take great pride and know that we are blessed to have her as a mentor, and hope we can one day make an impact to someone else the way she has done to us Thank you for being an awesome mentor.

Abstract

Background: This is a descriptive cross sectional study that conducted in Military Hospital during the period from June, 2022 to October 2022.

Aim: detect the variation of Prothrombin Time and Activated Partial Thromboplastin Time between diabetic patient attending Military Hospital for diabetes and healthy individual.50 samples were collected from both genders, 25 samples from diabetic patients in which there were 14 males and 11 females and 25 samples from healthy individuals in which there were 17 males and 8 females.

Material and method: In this cross sectional study case control study 25 diabetic patients and 25 healthy individuals were subjected to Prothrombin time(PT), Activated partial thromboplastin time(APTT) and international normalization ratio(INR) patients in the department of hematology, this study conducted in Military Hospital during the period from June to October 2022,

Results: the results showed that, the mean of prothrombin time was 15.440 sec in diabetic patients and 17.564 sec in healthy individuals, there is significant difference in PT (P=0.038). The mean of activated partial thromboplastin time was 33.868 sec in diabetic patients and 36.592 sec in healthy individuals, there is insignificant difference in APTT (P=0.336). The mean of INR was 1.7440 sec in diabetic patients and 1.6904 sec in healthy individuals there is insignificant difference in INR (P=0.649).

Conclusion: This mean that there is a significant association between Diabetes mellitus and coagulation parameters which may result in increased morbidity and premature mortality of diabetic patient.

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Abbreviations

ADP	:	Adenosine Diphosphate.
aPTT	:	activated partial thromboplastin Time.
cmm	:	cubic millimeter.
DCCT	:	Diabetes Control and Complications Trial.
DM	:	Diabetes Mellitus.
EDTA	:	Ethylene Diamine Tetra Acetic acid.
FFP	:	fresh frozen plasma.
g/dl	:	gram per deciliter.
GDM	:	Gestational Diabetes Mellitus.
GP	:	Glycoprotein.
Hb	:	hemoglobin.
HbA_{1C}	:	Glycated Hemoglobin.
Hct	:	hematocrit.
HMWK	:	high-molecular-weight kininogen.
IDDM	:	Insulin-Dependent Diabetes Mellitus.
INR	:	international normalized ratio.
ISI	:	international sensitivity index.

KCCT : kaolin-cephalin clotting time.

MCH : Mean Cellular Hemoglobin.

MCHC : Mean Cellular Hemoglobin Concentration.

MCV : Mean Cellular Volume.

Mmol/l : mill moles per liter.

Mmol/mol : mill moles per moles.

NIDDM : Non-Insulin-Dependent Diabetes Mellitus.

PAF : platelet- activating factor.

PCC : prothrombin complex concentrate.

PLA2 : activated phospholipase A2.

PR : prothrombin ratio.

PT : Prothrombin Time.

RBC : Red Blood Cell.

Sec : second.

SPSS : Statistical Package for the Social Sciences.

T1D : Type 1 Diabetes.

T2D : Type 2 Diabetes.

TF : tissue factor.

TFP1 : tissue factor pathway inhibitor.

TXA2 : thromboxane A2.

VWF : von willebrand factor.

WBC : White Blood Cell.

WHO : World Health Organization.

Chapter one

Introduction

1. Introduction

1.1 Diabetes mellitus (DM):

It is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. Diabetes is due to either the pancreas not producing enough insulin or the cell of the body not responding properly to the insulin produced. (David *et al*, 2011)

1.1.1 Types of diabetes mellitus:

There are three main types of diabetes mellitus:

-Type 1 DM results from the body's failure to produce enough insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". The cause unknown. (kitabchi *et al*, 2009)

-Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the progresses a lack of insulin may also develop. This was previously referred to as "non-insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes'. The primary cause is excessive body weight and not enough exercise. (kitabchi *et al*, 2009)

-Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level. (kitabchi *et al*,2009)

1.1.2 Sings and symptoms:

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger). Several other signs and symptoms can mark the onset of diabetes, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. (Cooke *et al*, 2008)

1.1.3 Complication of diabetes mellitus:

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease. The primary microvascular complications of diabetes include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness. Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant. Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. (O'Gara *et al*, 2013)

Table (1.1): Comparison of type 1 and 2 diabetes: (Sarwar *et al*, 2010)

Feature	Type 1 diabetes	Type 2 diabetes
Onset	**Sudden**	**Gradual**
Age of onset	**Mostly in children**	**Mostly in adults**
Body size	**Thin or normal**	**Often obese**
Ketoacidosis	**Common**	**Rare**
Autoantibodies	**Usually present**	**Absent**
Endogenous insulin	**Low or absent**	**Normal, decreased or increased**
Concordance in identical twins	**50%**	**90%**
Prevalence	**~10%**	**~90%**

1.1.4 Causes of diabetes mellitus:

Type 1 diabetes is partly inherited, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection or diet.

Type 2 diabetes is due primarily to lifestyle factors and genetics. A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, poor diet, stress.

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2-10% of all pregnancies and may improve or disappear after delivery. However, after pregnancy approximately 5-10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2. (Risérus *et al*, 2009)

1.1.5 Pathophysiology of diabetes mellitus:

Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, muscle, and adipose tissue. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. Insulin is released into the blood by beta cells (B-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. (Kim, 2012)

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose. When the glucose concentration in the blood remains high

over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria). (Robert, 2012)

1.1.6 Diagnosis of diabetes mellitus:

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following: (Lee *et al*, 2012)

. Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)

. Plasma glucose≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test

. Glycated hemoglobin (HbA1c) ≥ 48 mmol/mol (6.5 DCCT%).

Table (1.2): Who diabetes diagnosis criteria: (Kim, 2012)

Condition	2 hour glucose	Fasting glucose	HbA1c	
Unit	mmol/l(gm/dl)	mmol/l(gm/dl)	mmol/mol	DCCT%
Normal	<7.8(<140)	<6.1(<110)	<42	<6.0
Impaired fasting glycaemia	<7.8(<140)	>6.1(>110) & <7.0(<126)	42-46	6.0-6.4
Impaired glucose tolerance	>7.8(>140)	<7.0(<126)	42-46	6.0-6.4
Diabetes mellitus	>11.1(>200)	>7.0(>126)	>48	>6.5

1.2 Coagulation:

Coagulation, also known as clotting, is the process by which blood changes from a liquid to a gel, forming a blood clot. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves activation, adhesion and aggregation of platelets, as well as deposition and maturation of fibrin.

Coagulation begins almost instantly after an injury to the endothelium lining a blood vessel. Exposure of blood to the sub endothelial space initiates two processes: changes in platelets, and the exposure of sub endothelial tissue factor to plasma factor VII, which ultimately leads to cross-linked fibrin formation. Platelets immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously: additional coagulation (clotting) factors beyond factor VII (listed below) respond in a cascade to form fibrin strands, which strengthen the platelet plug. (Furie *et al*, 2005)

Coagulation is highly conserved throughout biology. In all mammals, coagulation involves both cellular components (platelets) and proteinaceous components (here, coagulation factors). [3] The pathway in humans has been the most extensively researched and is the best understood. (Schmaier *et al*, 2011)

1.2.1 Physiology:

1.2.1.1 Platelet activation:

When the endothelium is damaged, the normally isolated underlying collagen is exposed to circulating platelets, which bind directly to collagen with collagen-specific glycoprotein Ia/IIa surface receptors. This adhesion is strengthened further by von Will brand factor (vWF), which is released from the endothelium and from platelets; vWF forms additional links between the platelets' glycoprotein Ib/IX/V and A1 domain. This localization of platelets to the extracellular matrix promotes collagen interaction with

platelet glycoprotein VI. Binding of collagen to glycoprotein VI triggers a signaling cascade that results in activation of platelet integrins. Activated integrins mediate tight binding of platelets to the extracellular matrix. This process adheres platelets to the site of injury. (Michael Makris *et al*, 2009).

Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet-activating factor (PAF), vWF, platelet factor 4, and thromboxane A2 (TXA2), which, in turn, activate additional platelets. The granules' contents activate a GQ-linked protein receptor cascade, resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C, which, in turn, activates phospholipase A2 (PLA2). PLA2 then modifies the integrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelets change shape from spherical to stellate, and the fibrinogen cross-links with glycoprotein IIb/IIIa aid in aggregation of adjacent platelets (completing primary hemostasis). (Watson *et al*,2010)

1.2.1.2 Coagulation cascade:

The coagulation cascade of secondary hemostasis has two initial pathways which lead to fibrin formation. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor pathway (also known as the extrinsic pathway), which both lead to the same fundamental reactions that produce fibrin. It was previously thought that the two pathways of coagulation cascade were of equal importance, but it is now known that the primary pathway for the initiation of blood coagulation is the tissue factor (extrinsic) pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase a appended to indicate an active form. (Watson *et al*, 2010)

The coagulation factors are generally enzymes called serine proteases, which act by cleaving downstream proteins. The exceptions are tissue factor, FV, FVIII, FXIII. Tissue factor, FV and FVIII are glycoproteins, and Factor XIII is a transglutaminase. The coagulation factors circulate as inactive zymogens. The coagulation cascade is therefore classically divided into three pathways. The tissue factor and contact activation pathways both activate the "final common pathway" of factor X, thrombin and fibrin. (Hoffbrand *et al*, 2002)

1.2.1.2.1 Tissue factor pathway (extrinsic):

The main role of the tissue factor (TF) pathway is to generate a "thrombin burst", a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles is released very rapidly. FVIIa circulates in a higher amount than any other activated coagulation factor. The process includes the following steps. (Watson *et al*, 2010)

Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa). TF-FVIIa activates FIX and FX. FVII is itself activated by thrombin, FXIa, FXII, and FXa. The activation of FX (to form FXa) by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI). FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.

Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which forms a complex with FIX), and activates and releases FVIII from being bound to vWF.

FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues. (Long AT *et al*, 2016)

1.2.1.2.2 Contact activation pathway (intrinsic):

The contact activation pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK), prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that individuals with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder. Instead, contact activation system seems to be more involved in inflammation, and innate immunity. Despite this, interference with the pathway may confer protection against thrombosis without a significant bleeding risk. (Long AT *et al*, 2016)

1.2.1.2.3 Final common pathway:

The division of coagulation in two pathways is arbitrary, originating from laboratory tests in which clotting times were measured either after the clotting was initiated by glass, the intrinsic pathway; or clotting was initiated by thromboplastin (a mix of tissue factor and phospholipids), the extrinsic pathway.

Further, the final common pathway scheme implies that prothrombin is converted to thrombin only when acted upon by the intrinsic or extrinsic pathways, which is an oversimplification. In fact, thrombin is generated by activated platelets at the initiation of the platelet plug, which in turn promotes more platelet activation.

Thrombin functions not only to convert fibrinogen to fibrin, it also activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin); and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.

The coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex until it is down-regulated by the anticoagulant pathways. (waston *et al*,2010)

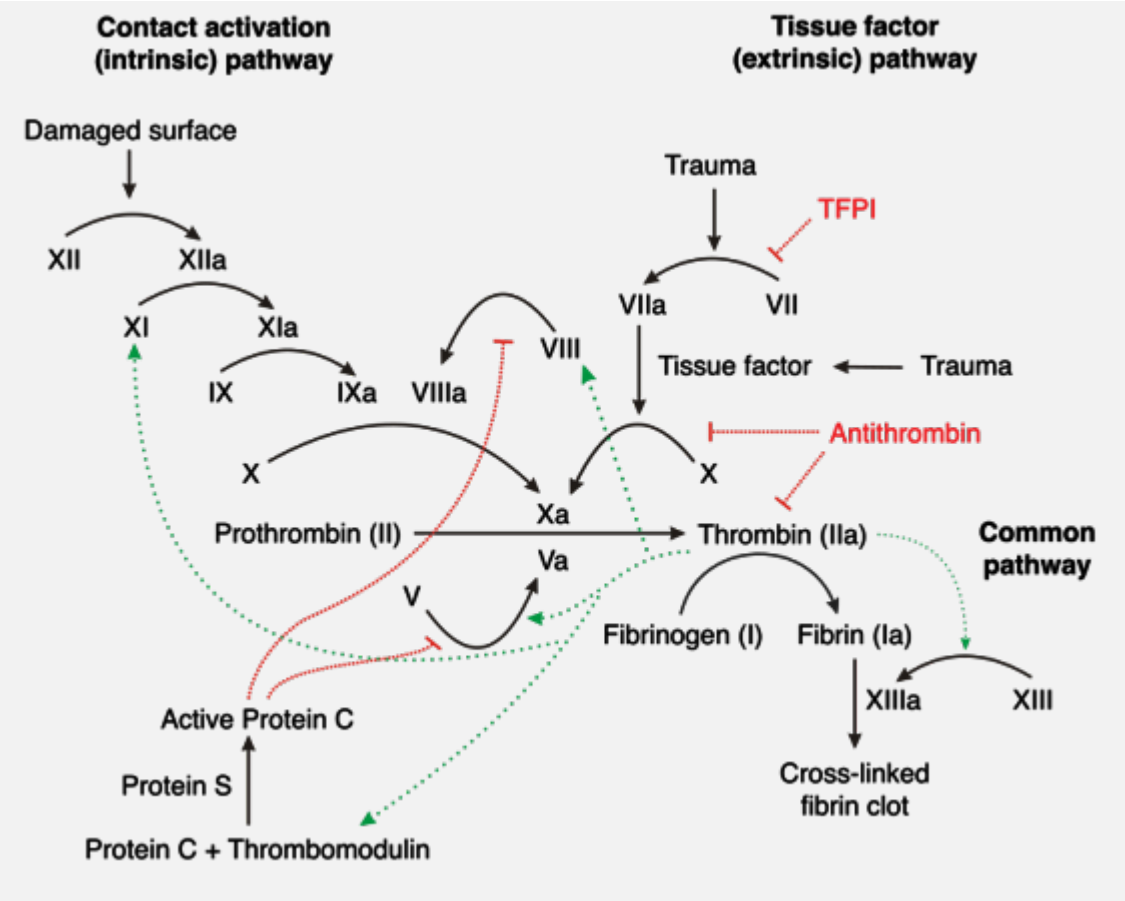

Fig :(1.1) coagulation cascade

1.2.2 Coagulation tests:

Coagulation tests measure your blood's ability to clot, and how long it takes to clot. Testing can help your doctor assess your risk of excessive bleeding or developing clots (thrombosis) somewhere in your blood vessels. Coagulation tests are useful in monitoring people who take medications that affect clotting ability. Coagulation tests

are also sometimes recommended before surgery. There are many types of coagulation tests:

- Prothrombin Time
- Activated Partial ThromboplastinTime
- Thrombin Time
- Platelets Count
- Bleeding Time

1.2.3 Prothrombin Time:

The prothrombin time (PT) – along with its derived measures of prothrombin ratio (PR) and international normalized ratio (INR) – is an assay for evaluating the extrinsic pathway and common pathway of coagulation. This blood test is also called protime INR and PT/INR. They are used to determine the clotting tendency of blood, in such things as the measure of warfarin dosage, liver damage, and vitamin K status. PT measures the following coagulation factors: I (fibrinogen), II (prothrombin), V (proaccelerin), VII (proconvertin), and X (Stuart–Prower factor).

PT is often used in conjunction with the activated partial thromboplastin time (aPTT) which measures the intrinsic pathway and common pathway of coagulation.

1.2.3.1 Laboratory Measurement of PT:

The reference range for prothrombin time depends on the analytical method used, but is usually around 12–13 seconds (results should always be interpreted using the reference range from the laboratory that performed the test), and the INR in absence of anticoagulation therapy is 0.8–1.2. The target range for INR in anticoagulant use (e.g. warfarin) is 2 to 3. In some cases, if more intense anticoagulation is thought to be required, the target range may be as high as 2.5–3.5 depending on the indication for anticoagulation. (Health, Ministry of. "BC Guidelines - Province of British Columbia")

1.2.3.2 Methodology of Prothrombin Time:

-Prothrombin time is typically analyzed by a laboratory technologist on an automated instrument at 37 °C (as a nominal approximation of normal human body temperature).

-Blood is drawn into a test tube containing liquid sodium citrate, which acts as an anticoagulant by binding the calcium in a sample. The blood is mixed, then centrifuged to separate blood cells from plasma (as prothrombin time is most commonly measured using blood plasma). In newborns, a capillary whole blood specimen is used. (Fritsma *et al*, 2002)

-A sample of the plasma is extracted from the test tube and placed into a measuring test tube (Note: for an accurate measurement, the ratio of blood to citrate needs to be fixed and should be labeled on the side of the measuring test tube by the manufacturing company; many laboratories will not perform the assay if the tube is underfilled and contains a relatively high concentration of citrate—the standardized dilution of 1-part anticoagulant to 9 parts whole blood is no longer valid).

-Next an excess of calcium (in a phospholipid suspension) is added to the test tube, thereby reversing the effects of citrate and enabling the blood to clot again.

-Finally, in order to activate the extrinsic / tissue factor clotting cascade pathway, tissue factor (also known as factor III) is added and the time the sample takes to clot is measured optically. Some laboratories use a mechanical measurement, which eliminates interferences from lipemic and icteric samples.

1.2.3.3 Prothrombin time ratio:

The prothrombin time ratio is the ratio of a subject's measured prothrombin time (in seconds) to the normal laboratory reference PT. The PT ratio varies depending on the specific reagents used, and has been replaced by the INR. Elevated INR may be useful as a rapid and inexpensive diagnostic of infection in people with COVID-19. (Bussey *et al*, 1992)

1.2.3.4 International normalized ratio:

The result (in seconds) for a prothrombin time performed on a normal individual will vary according to the type of analytical system employed. This is due to the variations between different types and batches of manufacturer's tissue factor used in the reagent to perform the test. The INR was devised to standardize the results. Each manufacturer assigns an ISI value (International Sensitivity Index) for any tissue factor they manufacture. The ISI value indicates how a particular batch of tissue factor compares to an international reference tissue factor. The ISI is usually between 0.94 and 1.4 for more sensitive and 2.0–3.0 for less sensitive thromboplastins. (Van Den Besselaar *et al*, 2004)

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

PT Normal is established as the geometric mean of the prothrombin times (PT) of a reference sample group. (D'Angelo A *et al*, 1997)

1.2.3.5 Interpretation of Prothrombin Time:

The prothrombin time is the time it takes plasma to clot after addition of tissue factor (obtained from animals such as rabbits, or recombinant tissue factor, or from brains of autopsy patients). This measures the quality of the extrinsic pathway (as well as the common pathway) of coagulation. The speed of the extrinsic pathway is greatly affected by levels of functional factor VII in the body. Factor VII has a short half-life and the carboxylation of its glutamate residues requires vitamin K. The prothrombin time can be prolonged as a result of deficiencies in vitamin K, warfarin therapy, malabsorption, or lack of intestinal colonization by bacteria (such as in newborns). In addition, poor factor VII synthesis (due to liver disease) or increased consumption (in disseminated intravascular coagulation) may prolong the PT.

The INR is typically used to monitor patients on warfarin or related oral anticoagulant therapy. The normal range for a healthy person not using warfarin is 0.8–1.2, and for people on warfarin therapy an INR of 2.0–3.0 is usually targeted, although the target INR may be higher in particular situations, such as for those with a mechanical heart valve. If the INR is outside the target range, a high INR indicates a higher risk of bleeding, while a low INR suggests a higher risk of developing a clot. In patients on a vitamin K antagonist such as warfarin with supratherapeutic INR but INR less than 10 and no bleeding, it is enough to lower the dose or omit a dose, monitor the INR and resume the vitamin K antagonist at an adjusted lower dose when the target INR is reached.[9] For people who need rapid reversal of the vitamin K antagonist – such as due to serious bleeding – or who need emergency surgery, the effects of warfarin can be reversed with vitamin K, prothrombin complex concentrate (PCC), or fresh frozen plasma (FFP).(Ageno W, 2012)

Further information: Warfarin § Overdose

1.2.3.6 Factors determining accuracy:

Lupus anticoagulant, a circulating inhibitor predisposing for thrombosis, may skew PT results, depending on the assay used. Variations between various thromboplastin preparations have in the past led to decreased accuracy of INR readings, and a 2005 study suggested that despite international calibration efforts (by INR) there were still statistically significant differences between various kits, casting doubt on the long-term tenability of PT/INR as a measure for anticoagulant therapy. Indeed, a new prothrombin time variant, the Fiix prothrombin time, intended solely for monitoring warfarin and other vitamin K antagonists has been invented and recently become available as a manufactured test. The Fiix prothrombin time is only affected by reductions in factor II and/or factor X and this stabilizes the anticoagulant effect and appears to improve clinical outcome according to an investigator initiated randomized blinded clinical trial, The Fiix-trial. In this trial thromboembolism was reduced by 50%

during long-term treatment and despite that bleeding was not increased.(Garlando AM *et al*, 1999)

1.2.4 Activated Partial Thromboplastin Time:

The partial thromboplastin time (PTT), also known as the activated partial thromboplastin time (aPTT or APTT), is a blood test that characterizes coagulation of the blood. A historical name for this measure is the kaolin-cephalin clotting time (KCCT), reflecting kaolin and cephalin as materials historically used in the test. Apart from detecting abnormalities in blood clotting, partial thromboplastin time is also used to monitor the treatment effect of heparin, a widely prescribed drug that reduces blood's tendency to clot.(KCCT, 2010)

The PTT measures the overall speed at which blood clots form by means of two consecutive series of biochemical reactions known as the intrinsic pathway and common pathway of coagulation. The PTT indirectly measures action of the following coagulation factors: I (fibrinogen), II (prothrombin), V (proaccelerin), VIII (anti-hemophilic factor), X (Stuart–Prower factor), XI (plasma thromboplastin antecedent), and XII (Hageman factor).

The PTT is often used in conjunction with another measure of how quickly blood clotting takes place called the prothrombin time (PT). The PT measures the speed of clotting by means of the extrinsic pathway and common pathway. (MedlinePlus Medical Encyclopedia, 2009)

1.2.4.1 Methodology of Activated Partial Thromboplastin Time:

-Partial thromboplastin time is typically analyzed by a medical technologist or a laboratory technician on an automated instrument at 37 °C (as a nominal approximation of normal human body temperature). The test is termed "partial" due to the absence of tissue factor from the reaction mixture.

-Blood is drawn into a test tube containing oxalate or citrate, molecules which act as an anticoagulant by binding the calcium in a sample. The blood is mixed, then centrifuged to separate blood cells from plasma (as partial thromboplastin time is most commonly measured using blood plasma).

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

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

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

1.2.4.2 Interpretation of Activated Partial Thromboplastin Time:

The typical reference range is between 30 seconds and 50 s (depending on laboratory). Shortening of the PTT is considered to have little clinical relevance, but some research indicates that it might increase risk of thromboembolism. (Korte, 2000) Normal PTT requires the presence of the following coagulation factors: I, II, V, VIII, IX, X, XI and XII. Notably, deficiencies in factors VII or XIII will not be detected with the PTT test.

Prolonged aPTT may indicate:

- use of heparin (or contamination of the sample)
- antiphospholipid antibody (especially lupus anticoagulant, which paradoxically increases propensity to thrombosis)
- coagulation factor deficiency (e.g., hemophilia)
- sepsis — coagulation factor consumption
- presence of antibodies against coagulation factors (factor inhibitors)

To distinguish the above causes, mixing tests are performed, in which the patient's plasma is mixed (initially at a 50:50 dilution) with normal plasma. If the abnormality does not disappear, the sample is said to contain an "inhibitor" (either heparin, antiphospholipid antibodies or coagulation factor specific inhibitors), while if it does disappear a factor deficiency is more likely. Deficiencies of factors VIII, IX, XI and XII and rarely von Willebrand factor (if causing a low factor VIII level) may lead to a prolonged aPTT correcting on mixing studies.

The aPTT is usually normal in pregnancy but tends to slightly decrease in late pregnancy. (Hellgren M, April 2003)

Rational:

Some statistics reveal that around 80% of people with diabetes have fatal consequences due to the formation of blood clots because High blood sugar levels cause abnormalities in the process of coagulation and increased stimulation to form blood clots, the ability of these blood clots to dissolve is also reduced.

So patients with diabetes mellitus considered to have a hypercoagulable state and are associated with increased risk of thrombosis therefor this study was designed to evaluate the coagulation profile (activated partial thromboplastin time and prothrombin time) in diabetic patients to analyze the correlations between gender and coagulation parameters in comparison with normal individual and also to give awareness about this complication.

Objectives:

General objective:

This study to Determinate the variation of prothrombin time and activated partial thromboplastin time between diabetic patients attending in Military Hospital and apparently healthy individuals in Khartoum state during the period from June to October 2022

Specific Objectives:

- To determine the variation of PT between diabetic patients and healthy individuals.
- To determine the variation of INR between diabetic patients and healthy individuals
- To determine the variation of APTT between in diabetic patients and healthy individuals.

Chapter two

Literature review

2. Previous Studies

Several studies have been carried out to determine the variation of prothrombin time and activated partial thromboplastin time between diabetic patients and healthy individuals. In such study show that APTT and PT were significantly shorter among patients with T2DM compared to those without (20.88 ± 5.19 v 31.23 ± 5.41, P =0.0001; and 11.03 ± 2.06sec v 14.46 ± 1.86, P=0.0001 respectively). INR was decreased among patients with T2DM compared to those without (0.83 ± 0.18 v 1.13 ± 0.17, P=0.0001). (Richard *et al*, 2017)

Similar study was done in which there was significant difference observed in coagulation profile (Fibrinogen, Prothrombin time, INR, APTT, platelet count) between control group andT1DM. (Mariappan *et al*, 2017)

other study showed that the mean and standard of PT, APTT, INR was (17.9± 3.8 sec, 32.6± 4.1 sec, 1.1± 0.3 respectively), this show that there was shortened prothrombin time, activated partial thromboplastin time, shortened PT and APTT might be useful haemostatic markers in diabetic patients, especially in those at high risk for thrombotic complication. (Abdelrhman et al, 2020)

Another study showed a stastically significant increase in Mean Prothrombin time (PT) levels of 17.48 in cases vs 14.52 in controls with a P value was 0.012. The Mean aPTT levels in cases was 48.12 and in controls was 30.56 with a P value was 0.001. this mean that There is a significant association between Type 2 Diabetes mellitus and coagulation parameters. (Shaffy *et al*, 2018)

Similar study showed that there was statistically significantly lower values; (P = 0.031) in prothrombin time (13.47 ± 0.96 v 14.06 ± 0.96s) and (P = 0.001) in activated partial thrombopalstin time (34,39 ± 2.17v 37.25 ± 1.82) when type 2 diabetics was compared

with control subjects. The decrease in prothrombin time and APTT showed that type 2 diabetics is a state of chronic low grade inflammation, increased oxidative stress and hypercoagulable state which may result in increase morbidity and premature mortality of type 2 diabetes. (Edward *et al*, 2019)

Chapter three

Materials and Methods

3. Material and method

3.1 Study Design:

This is a cross sectional study that involved diabetics and control subjects.

3.2 Study Area:

This study conducted in Military Hospital during the period from June to October 2022, to Compare Prothrombin Time and Activated Partial Thromboplastin Time between diabetic patient and healthy individual.

Khartoum state.

3.3 Study population:

The data and samples were collected from 50 volunteers, 25 samples from diabetic patients and 25 samples from healthy individuals include male and female with different age.

3.3.1 Inclusion criteria:

- Both genders
- From all age groups.
- Healthy individuals for control samples
- Diabetic patients for test samples

3.3.2 Exclusion criteria:

Exclude other chronic disease and other diabetic patients with abnormal coagulation profile.

3.4 Materials:

3.4.1 Reagents:

- Prothrombin time reagent (thromboplastin)
- Kaolin and phospholipid
- Thrombin reagent

3.4.2 Equipments:

- Gloves.
- Alcohol swab.
- Tri sodium citrate.
- Cotton.
- Centrifuge.
- Automatic pipette.
- Yellow tips.
- Plain container.
- Potassium oxalate tube.
- Sodium fluoride tube.

3.5 Sampling Technique and Sample Size:

Electromechanical clot detection systems measure a change in conductivity between two metal electrodes in plasma. The BBL Fibro meter was the first semi-automated instrument to be used routinely in the coagulation laboratory. The probe of this instrument has one stationary and one moving electrode.

3.6 Data collection and Analysis:

Data will be collected using structure interviewing questionnaire, which design to collect and maintain all information concerning each case examined.

3.7 Data management and statistical analysis:

Data was entered into Microsoft work and statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 20.0 for Microsoft Windows, 5th students' edition. Continuous variables were reported using mean and standard deviation, Bar chart, Pie chart and One Sample T Test used to analyze categorical variables. $P<0.05$ was considered statistically significant.

3.8 Ethical Considerations:

The objective and procedure had been fully explained to each participant and their agreement was documented in a consent form.

Chapter Four

Result

4.Result

This is a descriptive cross sectional study that conducted in Military Hospital during the period from June to October 2022, to Compare Prothrombin Time and Activated Partial Thromboplastin Time between diabetic patient and healthy individual.

50 samples were collected from both genders, 25(50%) samples from diabetic patients and 25 (50%) samples from healthy individuals, this shown in table (4.1) and figure (4.1).

Table (4.1): Frequency of groups among all individuals:

Group	Frequency	Percentage
Diabetic patients	25	50%
Healthy individuals	25	50%
Total	50	100%

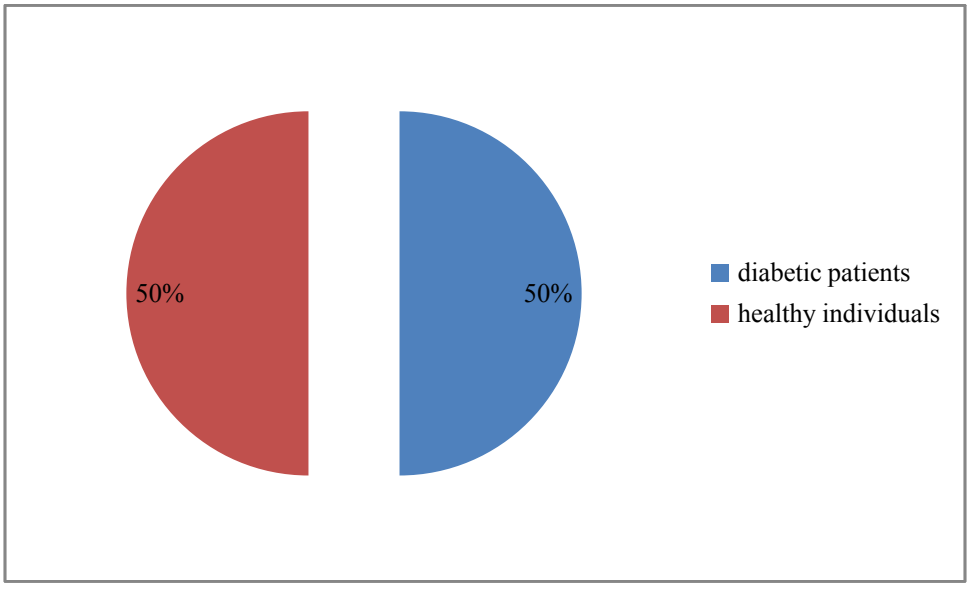

Figure (4.1): Frequency of group among all individuals

50 samples were collected from all individuals in which there were 19 (38%) females and 31 (62%) males, this shown in table (4.2) and figure (4.2).

Table (4.2): Frequency of gender among all individuals:

Gender	Frequency	Percentage
Female	19	38%
male	31	62%
Total	50	100%

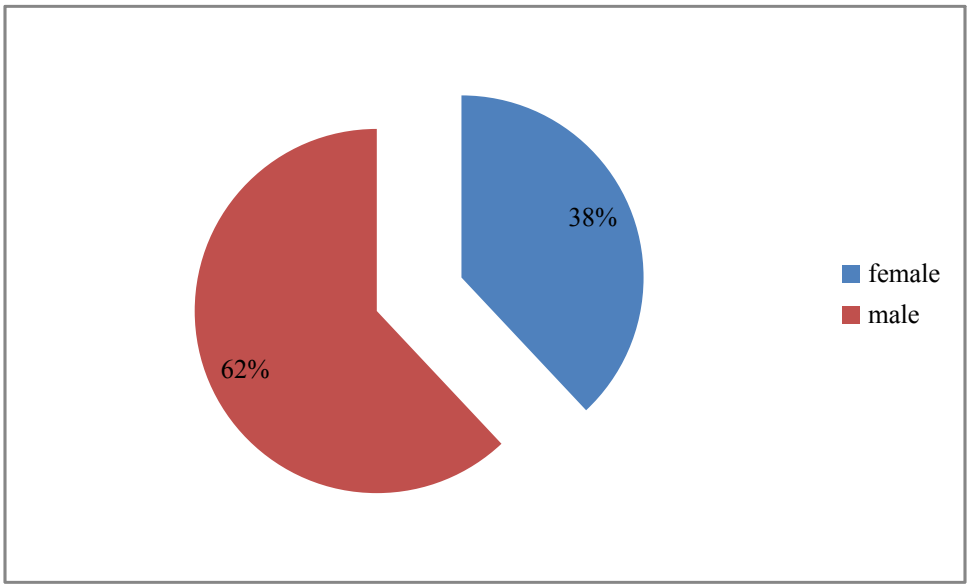

Fig :(4.2) Frequencies of Gender among all individuals

25 samples were collected from diabetic patient in which there were 11 (44%) females and 14 (56%) males, this shown in table (4.3) and figure (4.3).

Table (4.3): Frequency of gender among diabetic patients:

Gender	Frequency	Percentage
Female	11	44%
male	14	56%
Total	25	100%

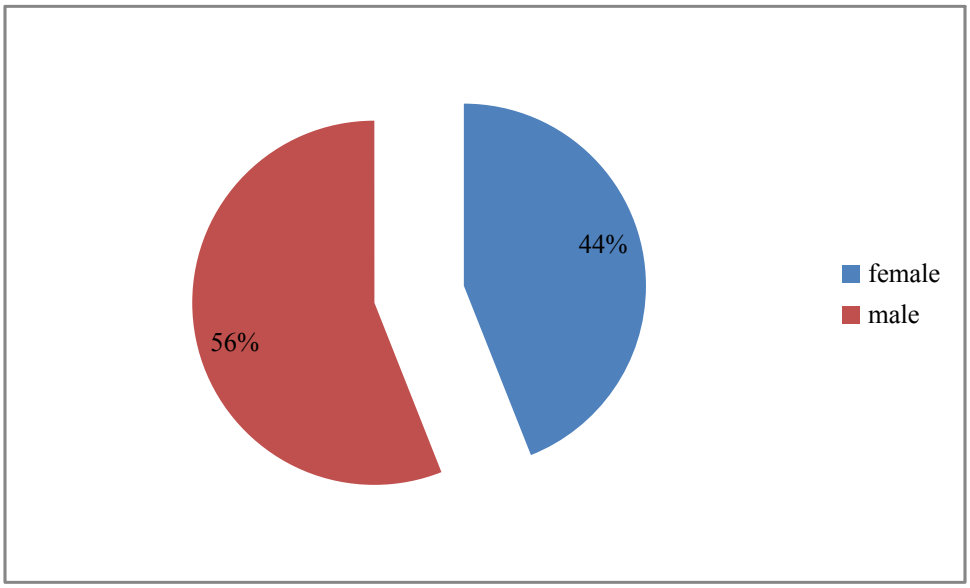

Figure (4.3): Frequency of gender among diabetic patients:

25 samples were collected from healthy individuals in which there were 8 (32%) females and 17 (68%) males, this shown in table (4.4) and figure (4.4).

Table (4.4): Frequency of gender among healthy individuals:

Gender	Frequency	Percentage
Female	8	32%
male	17	68%
Total	25	100%

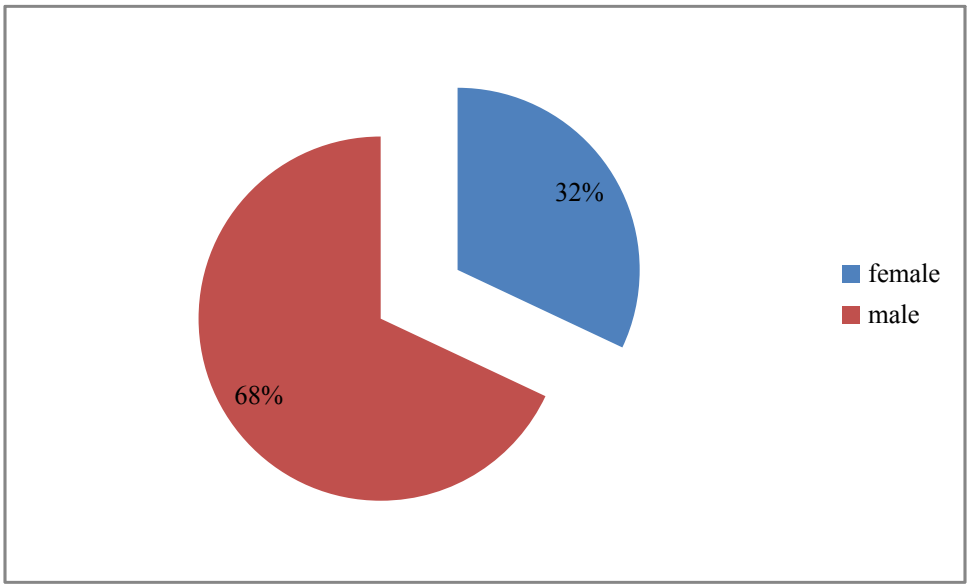

Figure (4.4): Frequency of gender among healthy individuals:

The mean of prothrombin time was 15.440 sec in diabetic patients and 17.564 sec in healthy individuals, this shown in table (4.5) and figure (4.5).

Table (4.5): PT in Diabetic patients and Healthy individuals:

	Diabetic patients	Healthy individuals	P-Value
PT (Mean)	15.440 sec	17.564 sec	0.038
The p-value is 0.038. The result is significant at $p < 0.05$.			

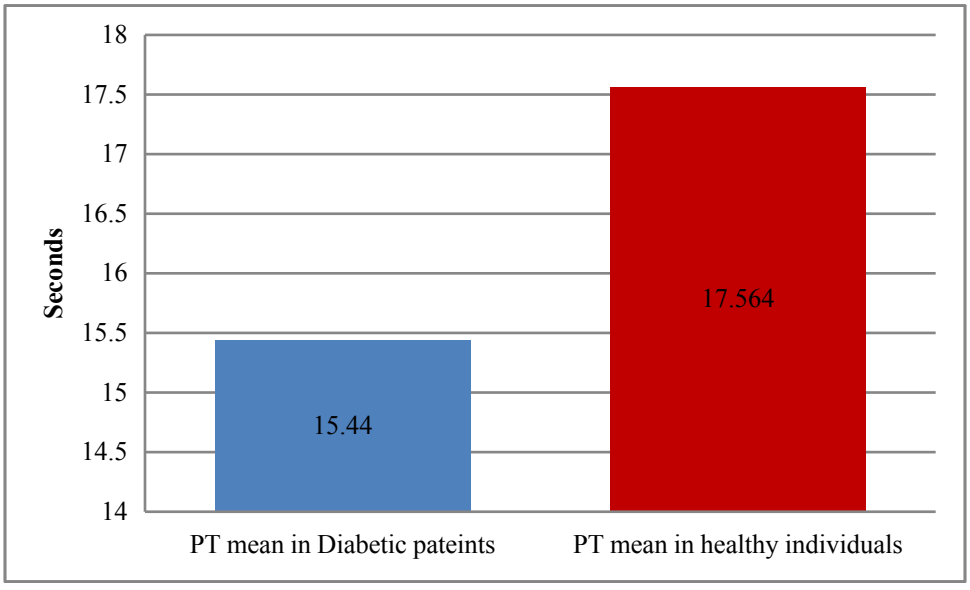

Figure (4.5): PT in Diabetic patients and Healthy individuals:

The mean of activated partial thromboplastin time was 33.868 sec in diabetic patients and 36.592 sec in healthy individuals, this shown in table (4.6) and figure (4.6).

Table (4.6): APTT in Diabetic patients and Healthy individuals:

	Diabetic patients	Healthy individuals	P-Value
APTT (Mean)	33.868 sec	36.592 sec	0.336
The p-value is 0.366. The result is significant at $p < 0.05$.			

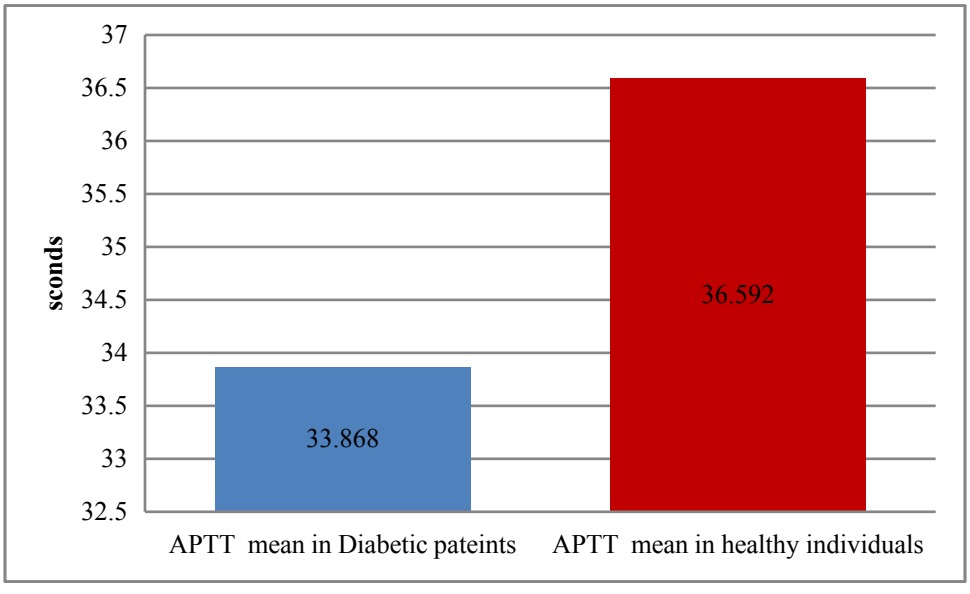

Figure (4.6): APTT in Diabetic patients and Healthy individuals:

The mean of INR was 1.7440 sec in diabetic patients and 1.6904 sec in healthy individuals, this shown in table (4.7) and figure (4.7).

Table (4.7): INR in Diabetic patients and Healthy individuals:

INR (Mean)	Diabetic patients	Healthy individuals	P-Value
	1.7440	1.6904	0.649
The p-value is 0.649. The result is significant at $p < 0.05$.			

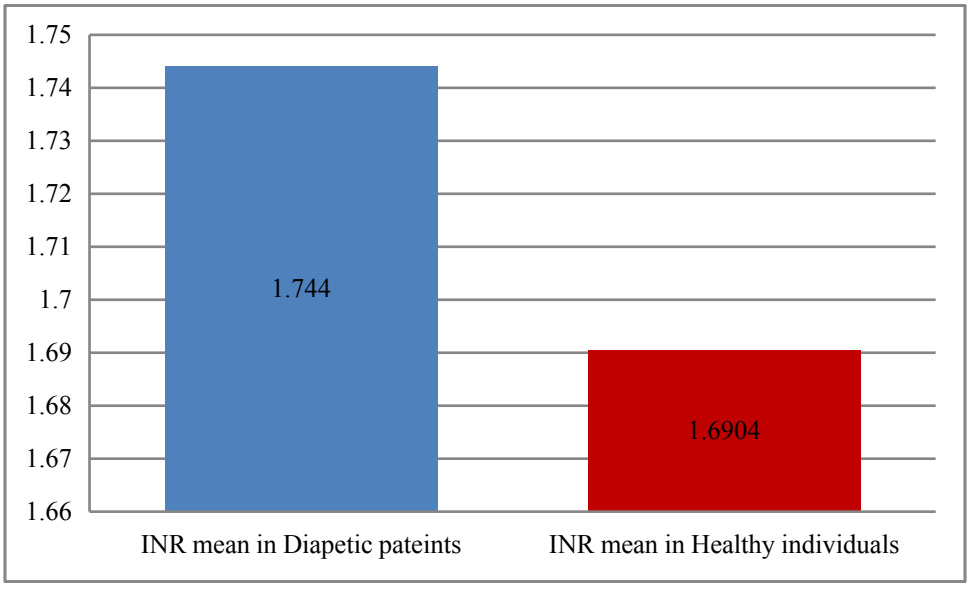

Figure (4.7): INR in Diabetic patients and Healthy individuals:

The mean of PT, APTT and INR in diabetic patients in comparison with healthy individuals is shown in table (4.8) and figure (4.8).

Table (4.8): Comparison of means of PT, APTT and INR in diabetic patient and healthy individuals:

Variable	Diabetic patients	Healthy individuals	P-Value
PT (mean)	15.440 sec	17.564 sec	0.038
APTT (mean)	1.7440	1.6904	0.336
INR (mean)	1.7440	1.6904	0.649
The result is significant at $p < 0.05$.			

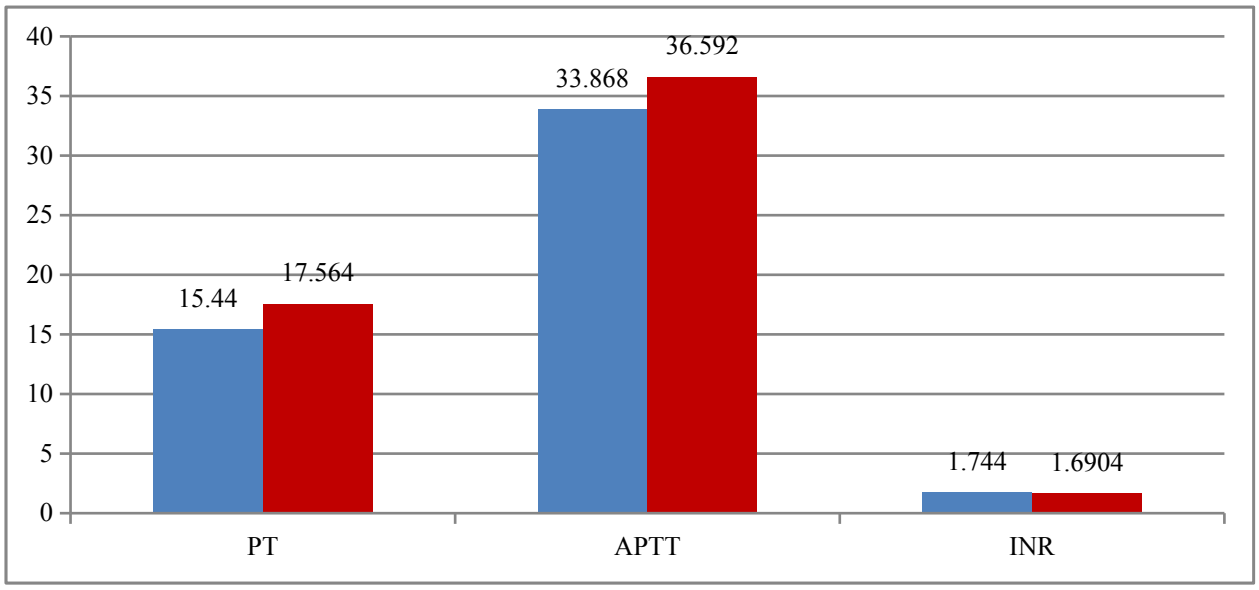

Figure (4.8): Comparison of means of PT, APTT and INR in diabetic patient and healthy individuals:

Chapter Five

Discussion

5. Discussion

This is a descriptive cross sectional study that conducted in Military Hospital during the period from June to October 2022, to detect the variation of Prothrombin Time and Activated Partial Thromboplastin Time between diabetic patient attending Military Hospital and healthy individuals. 50 samples were collected from both genders, 25 samples from diabetic patients in which there were 14 males and 11 females and 25 samples from healthy individuals in which there were 17 males and 8 females.

The mean of prothrombin time was 15.440 sec in diabetic patients and 17.564 sec in healthy individuals, there is significant difference in PT (P=0.038). This show similarity in comparison with other study in which the p value of PT was (P=0.0001). (Richard *et al*, 2017).Also show similarity in comparison with other study in which the p value of PT was (P = 0.031). (Edward *et al*, 2019)

The mean of activated partial thromboplastin time was 33.868 sec in diabetic patients and 36.592 sec in healthy individuals, there is insignificant difference in APTT (P=0.336). This show differently in comparison with other study in which the p value of APTT was (P=0.0001). (Richard *et al*, 2017).Also show differently in comparison with other study in which the p value of APTT was (P = 0.001). (Edward *et al*, 2019)

The mean of INR was 1.7440 sec in diabetic patients and 1.6904 sec in healthy individuals there is insignificant difference in INR (P=0.649). This show differently in comparison with other study in which the p value of INR was (P=0.0001). (Richard *et al*, 2017).

Chapter six

Conclusion and Recommendations

6. Conclusion and Recommendation

6.1 Conclusion:

- The Frequencies of diabetic patients were 25 while the Frequencies of healthy individual were 25.
- The Frequencies of gender in all individual were 19 females and 31 males.
- The Frequencies of gender in diabetic patients was 11 females and 14 males.
- The Frequencies of gender in healthy individual were 8 females and 17 males.
- The mean of PT in diabetic patients was 15.440 sec while the mean PT in healthy individual was 17.564 sec, in which there is significant difference in PT (P-value = 0.038).
- The mean of INR in diabetic patients was 1.7440 while the mean INR in healthy individual was 1.6904, in which there is insignificant difference in INR (P-value = 0.649).
- The mean of aPTT in diabetic patients was 33.868 sec while the mean aPTT in healthy individual was 36.592 sec in which there is insignificant differences in aPTT (P-value = 0.336).
- This study shows similarities in comparison with other study in which the PT in diabetic patients was lower than in healthy individual this highlighted the effect of diabetes on the coagulation cascade and coagulation tests.

6.2 Recommendation:

- Determination the variation of PT and aPTT between diabetic patients and healthy individuals in correlation to age, tribe, mediation and duration of disease.
- Determination the variation of PT and aPTT between diabetic patients and healthy individuals using larger samples and other geographical areas.
- Determination the variation of more coagulation profile test between diabetic patients and healthy individuals (e.g. Platelet count, PDW, MPV and thrombin time).
- Determination the variation of other hematological parameters between diabetic patients and healthy individuals (e.g. Hb, Hct, RBC, WBC, MCV, MCH, MCHC).

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