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Sudan University of Science and Technology

College of Graduate Studies

Estimation of Prothrombin Time, Activated Partial Thromboplastin Time among Hypertensive Patients in AL-Gezira State during period from March to May 2019

تقدير زمن البروثرومبين ، زمن الثرومبوبلاستين الجزئي النشط لدى مرضى إرتفاع ضغط الدم بولايه الجزيرة خلال الفترة من مارس الى مايو 2019

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الآية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى : -

﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

صَدَقَ اللَّهُ الْعَظِيمُ

سورة البقرة – الآية (32).

I

Dedication

To who taught me how to be available member?

In the community...

My father

To essence of life and meaning of humanity

My Mother

To who share with me all moments of happiness and sadness and made me

happy at time of sadness

'' My lovely family''

To who gave me sense of everlasting warmth and beauty?

''My best friends''

To all whom I love and respect,,

Acknowledgment

All thanks to Allah from the start to the end…..

And pray for Prophet Mohammed peace is upon him

I would like to acknowledge the contribution of my

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Who guide me throughout my way and helped me to make this research as accurate and useful as possible.

And I'm grateful to my friends and all those who contributed their time and helped me.

My thanks also extend to my college and my teachers

Abstract

This is a case control study to estimate Prothrombin Time, Activated Partial Thromboplastin Time among hypertensive patients in AL- Gezira state at the period from March to May 2019.

A total of 100 subjects were enrolled for this study, study population divided into case study group (n=50) who were hypertensive patients, and apparently healthy subjects as control group (n=50). Both, they're matched in age and sex. Venous blood sample (1.8ml) was collected in 3.2% tri sodium citrate container from each participant. Then, plasma was separated by

Centrifugations for 15 minutes, estimation of coagulation profile (PT, APTT) was performed by using coagulometer device (BT3505C192) and obtains data by (SPSS) program Version 20 was used for data analysis.

The result obtained from patient that the mean of PT, APTT in case were (14.3 sec), (30.1sec) respectively and compare to mean of PT, APTT were (13.2 sec), (29.7 sec) respectively in control with significant difference for PT (*p.value*=0.00) and no significant difference for APTT (*p.value*=0.71).

The study concluded that the prothrombin time is effected and Activated partial thromboplastin time is no effected by Hypertension.

المستخلص

هـذه دراسـة حالـه لقيـاس زمـن البروثـرومبين ، زمـن الثرمبوبلاسـتين الجزئـي النشـط لـدى مرضـى إرتفاع ضغط الدم بولاية الجزيره في الفترة من مارس إلى مايو2019

تـم تسـجيل مجمـوع 100شـخص فـي هـذه الدراسـة، و تـم تقسـيم مجتمـع الدراسـه الـي مجموعـة دراسـة الحالـة (ن=50) الـذين كـانوا مرضـى إرتفـاع ضـغط الـدم والمجموعـة التـي تبـدو صـحية كانـت المجموعـة الضـابطة (ن =50). كلاهمـا متطابقـان فـي العمـر والجنـس. جمعـت عينـة مـن الـدم الوريـدي (1.8 مـل) فـي حاويـة سـترات الصـوديوم 3.2 %. بعـد ذلـك ، تـم فصـل البلازمـا بـالطرد المركـزي لمـدة 15 دقيقـة ، وتقيـيم (PT,APTT) باسـتخدام جهـاز التخثـر (BT3503C192) تـم اسـتخدام برنـامج الحـزم الاحصـائيه الإصدار20 لتحليل البيانات.

المتوسـط الحسـابي لكـل مـن(PT,APTT) فـي الحالـه كـان (14.3ثانيـه)،(30.1 ثانيـه)على التـوالي مقارنـه بالمتوسـط الحسـابي لكـل مـن(PT,APTT) كـان(13.2 ثانيـه)،(29.7 ثانيـه) علـى التـوالي فـي المجموعـه الضـابطه مـع وجـود دلالـه احصـائيه لـزمن البرثـرومبين($p.value=0.00$)و عـدم وجـود دلاله احصائيه لزمن الثرمبوبلاستين الجزئي النشط($p.value=0.71$).

اثبتـت التحاليـل ان ضـغط الـدم يـؤثر علـى زمـن الثـرومبين ولا يـؤثر علـى زمـن الثرومبوبلاسـتين الجزئـي النشط.

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List of abbreviations

Abbreviation

ADP	Adenine Di Phosphate
Ag	Antigen
APTT	Activated Partial Thromboplastin Time
Ca^{++}	Calcium
DVT	Deep Vein Thrombosis
FDPs	Fibrin Degradation Products
GP	Glycoprotein
HMWK	High Molecular- Weight Kininogen
HTN	Hypertension
INR	International Normalization Ratio
MW	Molecular Wight
P.Value	Probability Value
PAI-1	Plasmin-Plasminogen Activator Inhibitor 1
PC	Protein C
PE	Pulmonary Embolism
PS	Protein S
PT	Prothrombin Time
SD	Stander Deviation
SPSS	Statistical Package for Social Science
TFPI	Tissue Factor Pathway Inhibitor
TPA	Tissue Plasminogen Activator
TT	Thrombin Time
UPA	Urokinase likes Plasminogen Activator
VWF	Von Willebrand Factor

Chapter One

1. Introduction

1.1. Coagulation

  Blood coagulation is a complex process by which blood forms clots. It is an important part of homeostasis, where in a damaged blood vessel wall is covered by a platelet and fibrin containing clot to stop bleeding and begin repair of the damage vessel(Ochei,2008). The several important functions of homeostasis included: maintain blood in a fluid state while it remains circulating within the vascular system, also arrest bleeding at the site of injury or blood loss by formation of a haemostatic plug, also limit process to the vicinity of the damage, and to ensure the eventual removal of the plug whilst healing is complete(Bain*etal*,2017). Thrombosis often appears to complicate the Coagulation begins almost instantly after an injury to the blood vessel has damages the endothelium. Exposure of the blood to protein such has tissue factors initiate changes to blood platelet and the plasma protein fibrinogen a clotting factor. Platelets immediately form a plug at the side of injury; this is called primary haemostasis.Secondary haemostasis occurs simultaneously; protein in the blood plasma called coagulation factors or clotting factors, respond in a complex cascade to form fibrin strands, which strengthen plug(Furie,2005).

Thrombosis in some patient with hypertension could be organ damage which makes a dramatic difference to clinical outcome in hypertension. The APTT is a screening test of the intrinsic clotting system. It will detect the inhibition or deficiency of one or more of the following factors: prothrombin, V, VIII (antihaemophilic factor), IX, X, XI, XII and fibrinogen.The PT is a screening test for the extrinsic clotting system, i.e. factor VII. It will also detect deficiencies of factors, prothrombin, V, X, and fibrinogen (Hoffbrand *et al*, 2008).

1.2. Hypertension

Hypertension is the term used to describe high blood pressure. Blood pressure is a measurement of the force against the walls of your arteries as the heart pumps blood through the body, Blood pressure readings are measured in millimeters of mercury (mmHg) and usually given as two numbers. Hypertension, or high blood pressure, is a very common and serious condition that can lead to or complicate many health problems. The risk of cardiovascular morbidity and mortality is directly correlated with blood pressure. Risks of stroke, MI, angina, heart failure, kidney failure or early death from a cardiovascular cause are directly correlated with BP (Luma and Spiotta, 2006).

Blood pressure is usually classified based on the systolic and diastolic blood pressures. Systolic blood pressure is the blood pressure in vessels during a heartbeat. Diastolic blood pressure measurement higher than the accepted normal value for the age of the individual is classified as pre-hypertension or hypertension (Chobanian *et al,* 2003).

High blood pressure is a trait as opposed to a specific disease and represents a quantitative rather than a qualitative deviation from the normal. Any definition of hypertension is therefore arbitrary. It is also known that hypertension is highly heritable and polygenic (caused by more than one gene) and a few candidate genes have been postulated in the etiology of this condition (Pimenta and Oparils, 2009).

Hypertension is often called "the silent killer" because it generally has no symptoms until serious complications develop. There are three general types of hypertension. Essential or primary hypertension occurs when the condition has no known cause. This form of hypertension cannot be cured, but it can be controlled. More than 90% of individuals with hypertension have essential hypertension. Fewer than 10% of patients have secondary hypertension; where either a co-morbid disease or drug is responsible for elevating BP. In most of these cases renal dysfunction resulting from sever chronic kidney disease or endovascular disease is the most common secondary cause (Giacchetti, 2009).

2. Rationale

Hypertension is worldwide disease that affects many people in different age. Systemic blood pressure rises with age, and the incidence of cardiovascular disease (Ramnik, 2010).

I want to conduct this study because the frequency of hypertension in Sudan is progress recently, and the high blood pressure affect on coagulation pathway, and we want to estimate of prothrombin time, Activated partial thromboplastin time among hypertensive patients.

3. Objectives

3.1 General objective

To estimate (PT, APTT) among hypertensive patients.

3.2 Specific objectives

- To measure(PT, APTT) in hypertensive patients and control group.

-To compare(PT, APTT) of hypertensive patients with normal control.

-To compare (PT, APTT) According to (Age, sex, Aspirin intake, other disease and duration of disease).

Chapter Two

Literature review

1. Hemostasis

Normal hemostatic 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. Never the less, such as response needs to be tightly controlled to prevent extensive clots developing and to break down such clots once damage is repaired. The hemostatic 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 (Beck, 2009).

1.1 Primary hemostasis

Primary haemostasis results from complex interactions between platelets, vessel wall and adhesive proteins leading to the formation of initial 'platelet plug', The endothelial cells lining the vascular wall exhibit the antithrombotic properties due to multiple factors viz: negatively charged heparin like glycosaaminoglycans, neutral phospholipids, synthesis and secretion of platelet inhibitors, coagulation inhibitors and fibrinolysis activators, In contrast, subendothelial layer is highly thrombogenic and contains collagen, Von Willebrand factor (VWF) and other proteins like laminin, thrombospondin and vitronectin that are involved in platelet adhesion, Any vascular insult results in arteriolar vasospasm, mediated by reflex neurogenic mechanisms and release of local mediators like endothelia and platelet derived thromboxane A2 (TxA2) (Beck ,2009).

Platelets

Platelet production

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytic, one of the largest cells in the body. The precursor of the megakaryocytic -the megakaryoblast-arises by a process of differentiation from the haemopoietic stem cell the megakaryocytic matures active, by end mitotic synchronous replication enlarging the cytoplasm 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 (Hoff brand, 2006).

Platelet function

The main steps in platelet functions are adhesion, activation with shape change and aggregation, When the vessel wall is damaged, the subendothelial structures, including basement membrane, collagen and microfibrils, are exposed (Bain *et al*,2017).

VWF binds to collagen and microfibrils and then captures platelets via initial binding to platelet GPIb, resulting in an initial monolayer of adherent platelets, Binding via GPIb initiates activation of the platelet via a G-protein mechanism Once activated, platelets immediately change shape from a disc to a sphere with numerous projecting pseudopods, After adhesion of a single layer of platelets to the exposed subendothelium, and stick to one another to form aggregates. Fibrinogen, fibronectin, further VWF released from platelets and the glycoprotein IbIX and IIbIIIa complexes are essential at this stage to increase the cell-to-cell contact and facilitate aggregation (Ciesla, 2007).

Role of platelets in homeostasis

Adhesion and aggregation forming the primary haemostatic plug, Clot retraction, Release of platelet activating and procoagulant molecules, Provision of a procoagulant surface for the reactions of the coagulation system (Munker *et al*, 2007).

Platelet adhesion

After vascular injury VWF acts as a bridge between endothelial collagen and platelet surface receptors GpIb and promotes platelet adhesion, The platelet glycoprotein complex I (GP‑Ib) is the principal receptor for VWF (Turgeon,2001).

Von will brand factor'(VWF)

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 chromosome 12 and is synthesized both in endothelial cells and megakaryocytes, and stored in Weiberl-Palade bodies and platelet 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 raise 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 multiversity, the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to numeric VWF and smaller multiverse by the specific plasma metalloprotease, ADAMTS-13 (Turgeon, 2001).

Platelet secretion:

After adhesion, degranulation from both types of granules takes place with the release of various factors, Release of calcium occurs here, Calcium binds to the phospholipids that appear secondary to the platelet activation and provides a surface for assembly of various coagulation factors (Rendu and Brohard, 2001).

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/IIIa receptors, which do not bind fibrinogen, VWF or other ligands. Stimulation of a platelet leads to an increase in GPIIb/IIIa molecules, due to binding of alpha-granule membrane (rich in receptors) with the plasma membrane, activation of surface-exposed GPIIb/IIIa, enabling platelet cross-linking with fibrinogen bridges. Binding brings about molecular conformational changes resulting in a firm connection and further activation of the platelet (Hoff brand, 2006).

1.2 Secondary hemostasis:

Secondary hemostasis 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 endothelialcells. When the factors are in a precursor form, the enzyme or zymogene is converted to an active enzyme or a protease (Beutler *et al*, 2010).

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 quickly (Lippi and Favaloro, 2018).

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 (Levrat *et al,* (2008).

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

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, 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, the contact group: Factor XI, factor XII, pre kallikrein, and high-molecular-weight kininogen (HMWK) are involved in the intrinsic pathway, moderately stable, and not consumed during coagulation (Turgeon, 2001).

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.

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.

Factor III, Thromboplastin**:** Tissue factor activates factor VII when blood is exposed to tissue fluids.

Factor IV, Ionized Calcium**:** This active form of calcium is needed for the activation of thromboplastin and for conversion of prothrombin to thrombin.

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.

Factor VI, Nonexistent:

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.

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.

Factor IX, Plasma Thromboplastin Component**:** 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

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.

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.

Factor XII, Hageman factor**:** This surface contact factor is activated by collagen. Patients do not bleed but have a tendency to thrombosis.

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

High-Molecular-Weight Kininogen:

This surface contact factor is activated by kallikrein.

Prekallikrein, Fletcher Factor:

This is a surface contact activator, in which 75% is bound to HMWK

Phases of Coagulation:

 Coagulation can be divided into three separate phases: *1*) An initiation phase, in which low amounts of active coagulant factors are generated; *2*) An amplification phase, in which the level of active coagulation factors is boosted; and*3*) A propagation phase, in which coagulation factors

bind to highly procoagulant membranes of activated platelets and fibrin clots are formed and extrinsic pathway is still widely used (Versteeg *et al*, 2013).

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.

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 (Hoff brand, 2006).

Intrinsic pathway:

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.

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.

1.3 Common pathway

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.

1.4 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

Proteolysis*:* Protease enzyme thrombin cleaves fibrinogen resulting in a fibrin monomer, A and B fibrin peptide.

Polymerization*:* This occurs spontaneously due to fibrin monomer that line up end-to-end due to hydrogen bonding.

Stabilization*:* This occurs when the fibrin monomers are linked covalently by XIIIa into fibrin polymers forming an insoluble fibrin clot (Hoff brand, 2006).

Fig1.1: shows coagulation cascade

Laboratory testing looks at the in vitro effect of the coagulation process which is measured by the PT,APTT, TT, fibrin degradation products (FDPs), and D-dimmer. 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 (Bhaskar *et al*, 2016).
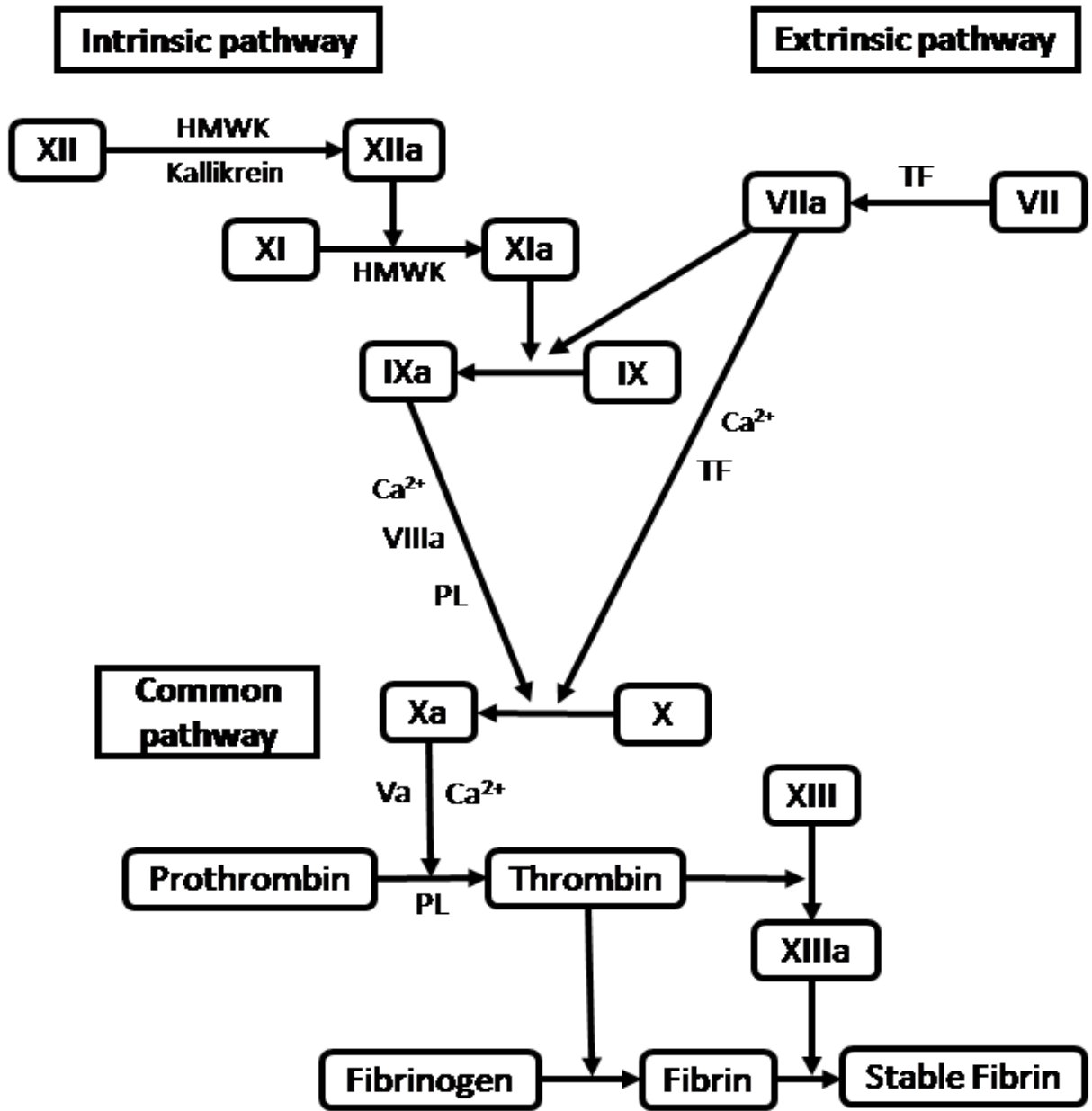
1.5Fibrinolysis system:

Fibrinolysis is the process in it plasmin degraded the fibrin. Following injury, TPAand urokinase-like plasminogen activator (UPA) released from damaged or activated cells, or exogenous agents, e.g. streptokinase, or therapeutic TPA or UPA, it activate plasminogen and convert it to plasmin, It digests fibrin (or fibrinogen) into fibrin degradation products (FDPs) and also degrades factors V and VII, Free plasmin is inactivated by plasma α2 antiplasmin and α2 macroglobulin (Mehta and Hoffbrand, 2005).

The clot or "thrombus" forms, circulating red blood cells, white blood cells, and platelets become incorporated into its structure. In addition, fibrin becomes cross-linked through the action of factor XIIIa, which is also activated by thrombin, and provides further structural stability (Bagoly *et al*, 2012).

1.6Coagulation 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

These inhibitors are:

Protease inhibitors: inhibitors of coagulation factors, which include (Antithrombin,Heparin cofactor II,Tissue factor pathway inhibitor, Alpha-2-antiplasmin, C1

The protein C pathway: inactivation of activated cofactors, which include (Protein C and protein S)

2. Hypertension

Hypertension (HTN) or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated. It is the opposite of hypotension. Hypertension is classified as either primary (essential) hypertension or secondary hypertension; About90–95% of cases is categorized as "primary hypertension," which means high blood pressure with no obvious medical cause. The remaining 5–10% of cases (Secondary hypertension) is caused by other conditions that affect the kidneys, arteries, heart or endocrine system (Carretero and Oparil, 2000).

2.1 Classification of hypertension:

Blood pressure is usually classified based on the systolic and diastolic blood pressure .A systolic or the diastolic blood pressure measurement higher than the accepted normal values for the age of individual is classified as prehypertension or hypertension.

Hypertension has several sub-classifications including, hypertension stage I, hypertension stage II, and isolated systolic hypertension. Isolated systolic hypertension refers to elevated systolic pressure with normal diastolic pressure and is common in the elderly. Individuals older than 50 years are classified as having hypertension if their blood pressure is consistently at least 140mmHg systolic or 90 mmHg diastolic .patient with blood pressures higher than 130mmHg with concomitant presence of diabetes mellitus or kidney disease require further treatment. Hypertension is also classified as resistant if medications do not reduce blood pressure to normal level (Chobanian *et al*, 2003).

2.2 Causes of hypertension:

The majority (80-90%) of patients with hypertension have primary elevation of blood pressure (i.e. cause not known - essential hypertension), which can be ameliorated only by life-long pharmacological therapy.

2.2.1Essential hypertension:

Essential hypertension is the most prevalent hypertension type ,Although no direct cause has been identified, there are many factors such as sedentary lifestyle ,smoking ,stress , visceral obesity ,potassium deficiency (Kyrou *et al*,2006) obesity (more than 85% of cases occur in those with a body mass index greater than 25), salt sensitivity .(Lackl ,2007).

Alcohol intake and vitamin D deficiency that increase the risk of developing hypertension. Risk also increases with aging, some inherited genetic mutations, and having a family history of

hypertension .An elevated level of rennin, a hormone secreted by the kidney, is another risk factor, as is sympathetic nervous system over activity (Rahmouni *et al*, 2005). Insulin resistance, which is a component of syndrome X (or metabolic syndrome), is also thought to contribute to hypertension .Recent studies have implicated low birth weight as a risk factor for adult essential hypertension. (Uchiyama, 2008).

2.2.2Secondary hypertension:

Secondary hypertension by definition results from an identifiable cause. This type is important to recognize since it's treated differently to essential hypertension, by treating the underlying cause of the elevated blood pressure. Hypertension results in the compromise or imbalance of the path physiological mechanisms, such as the hormone-regulating , that regulate blood plasma volume and heart function(Lee *et al,* 2010).

2.3 Signs and symptoms of hypertension:

In essential hypertension Mild to moderate essential hypertension is usually asymptomatic. In Secondary hypertension some additional signs and symptoms suggest that the hypertension is caused by disorders in hormone regulation. Hypertension combined with obesity distributed, accumulated fat on the back of the neck, wide purple marks on the abdomen, or the recent onset of diabetes suggests that an individual has a hormone disorder (Lee *et al*, 2010).

signs and symptoms associated with growth hormone(Khandwala and Hasnain,2009) And also cause of mental pressure in accelerated hypertension . Hypertensive encephalopathy is caused by severe small blood vessel congestion and brain swelling, which is reversible if blood pressure is, lowered (Ostchega *et al.,* 2008).

In Children**:** Some signs and symptoms are especially important in newborns and infants such as failure to thrive, seizures, irritability, lack of energy, and difficulty breathing. In children, hypertension can cause headache, fatigue, blurred vision, nose bleeds, and facial paralysis. Even with the above clinical symptoms, the true incidence of pediatric hypertension is not known. In adults, hypertension has been defined due to the adverse effects caused by hypertension. However, in children, similar studies have not been performed thoroughly to link any adverse effects with the increase in blood pressure. Therefore, the prevalence of pediatric hypertension remains unknown due to the lack of scientific knowledge (Rodriguez *et al.,* 2010).

2.4 Pathology of hypertension:

Most of the mechanisms associated with secondary hypertension are generally fully understood. However, those associated with essential hypertension are far less understood. What is known is that cardiac output is raised early in the disease course, with total peripheral resistance (TPR) normal; over time cardiac output drops to normal levels but TPR is increased(Nicholas *et al,* 2007).

2.5 Diagnosis:

Hypertension is generally diagnosed on the basis of a persistently high blood pressure. Usually this requires three separate sphygmomanometer measurements at least one week apart. Often, this entails three separate visits to the physician office .Initial assessment of the hypertensive patient should include a complete history and physical examination. Exceptionally, if the elevation is extreme, or if symptoms of organ damage are present then the diagnosis may be given and treatment started immediately.

Once the diagnosis of hypertension has been made, physicians will attempt to identify the underlying cause based on risk factor and other symptoms, if present, secondary hypertension is more common in preadolescent children, with most cases caused by renal disease. Primary or essential hypertension is more common in adolescents and has multiple risk factors (luma and Spiotta. 2006).

Laboratory tests can also be performed to identify possible causes of secondary hypertension and determine if hypertension has caused damage to heart, eyes, and kidneys.

Additional tests for Diabetes and high cholesterol levels are also usually performed because they are additional risk factors for the development of heart disease require treatment (Pierdomenico, et *al* .2009).

2.6 Complication of hypertension:

Hypertension is the most important risk factor for death in industrialized countries it increases hardening of the arteries (Calhoun.*et al*, 2008) and possible complications include: Aortic dissection, Blood vessel damage, Congestive heart failure, chronic kidney disease, chronic heart disease, Heart attack disease, Pregnancy complication (eclampsia), Hypertensive heart disease, Hypertensive retinopathy and Peripheral artery disease (Singer, 2008).

2.7 Previous Studies

In Nigeria 2017 Jiskani and his colleges evaluated PT, APTT in newly diagnosed Hypertensive patients .The results obtained showed that mean±SD of PT for the test subject was15.1 ± 1.92 in compared with the control subject showed a mean value 12.36 ± 0.74. While the result obtained from APTT test subject showed a mean value of 37.14± 4.06compared with the control subject that have a mean value of 30.4 ± 2.4.and result obtained from INR for test was 1.04±0.18 compared with control subject that have 0.87±0.07 When these results were compared statistically significant difference were observed PT (P=0.01)which indicate a state of prolongation in PT and APTT (Jiskani, 2017).

In Yenagoa 2018 Eeldo and his colleges PT, APTT and platelets count among hypertensive patients. The result for hypertensive patients and control subjects for PT were 15.49sec and 11.79 sec respectively, Aptt were 35.47sec and 28.57respectively (Eeldo *et al, 2018*).

Sechi 2000 the study relationship of fibrinogen level and haemostatic with organ damage in hypertension .the results showed significantly greater in hypertensive patients than normotensive control and were positively correlated with blood pressure (Sechi *et al*, 2000).

In khartoum2007 Fathelrahman study the haemostatic abnormalities and renal damage in Sudanese hypertensive patients, The results showed no significant difference between mean level of patients and controls in PT (p=0.626), APTT (p=0.272) (Fathelrahman, 2007).

Chapter Three

Materials and Methods

3.1Study design

This is case control and hospital based study.

3.2Study area and duration

The study was conducted in Aldefetein health centre which located in Gazira state in Sudan, during the period between March2019 to May2019.

3.3Study population

Study group were hypertensive patients and control group were healthy individuals.

3.3.1 Inclution Criteria

Individual of both sexes were hypertensive, were included in the study.

3.3.2Exclusion Criteria

Individual whom have previous history of thrombosis and undertake of warfarin or heparin therapy were excluded from study.

3.3.3Sampling

Individual were diagnosed with hypertension were selected and data collected using self-administrated per-coded questionnaire which was specifically designed to obtain information that helped in study.

3.3.4Sample Size

This study included 50 cases and 50 control individuals.

3.4 Data collection tools

Data were collected using questionnaires. This questionnaire was specifically designed to collect information about age, sex, other disease, aspirin intake and duration of this disease.

3.5Methodolgies

3.5.1 Blood sample collection and processing

Venous blood collected using sterile disposable plastic syringe after cleaning the venipuncture area with 70% ethanol, the blood was add to the anticoagulant at ratio of 4.5ml to .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 tubes, capped and frozen at -70°C used for PT, APTT.

3.5.2 Principle prothrombin time (PT):

The PT test measures the clotting time of re calcified plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system (Bain *et al* ,2011).

3.5.3Assay procedure:

Cuvettes were placed in incubation area for pre warming at 37c for 3 minute at least. 100 ml of pre warmed (37c) control or patient PPP was dispensed in cuvette in incubation area. Then cuvettes transferred to test area and 200ul of well mixed calcified thromboplasin reagent were added to cuvette, the analyzer timer started automatically when reagent was added. When clot formed timer was stopped automatically as result of OD changes, the analyzer is bi channel, get the mean of the two tested cuvettes and express it as PT on instrument display screen per seconds.

3.5.4Activated partial thromboplastin time (APTT):

3.5.5Principle of APTT:

The APTT was performed by automated testing in the batch or state mode. in the APTT an aliquot of undiluted, platelet poor plasma was incubated at 37c° with a particulate factor XII activator(i.e. ,silica, celite, kaolin ,ellagicacid ,etc. A reagent containing phospholipids (partial thromboplastin) was added, followed by $CaCl_2$ .the time required for clot formation after the addition of $CaCl_2$ .it measure over all activity of in intrinsic pathway (Bain *et al*,2017).

3.5.6Assay procedure:

Cuvettes were placed in incubation area for pre warming at 37c for 3 minute at least. 100 ml op pre warmed (37c) control or patient PPP was dispensed in cuvette in incubation area.

After 3 minute incubation 100ul of calcium chloride were added to each cuvette after they transferred to test area.

When clot formed timer was stopped automatically as result of OD changes, the analyzer is bi channel, get the mean of the two tested cuvettes and express it asAPTT on instrument display screen per seconds.

3.6 Ethical Considerations:

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

3.7Data analysis:

The statistical analysis of the results was performed by using the Statistical Package for Social Sciences (SPSS) using one independent T-test and correlation for testing significant and frequencies.

Chapter four
4. Results

One hundred volunteers were enrolled in this study classified to 50 hypertensive patients as case while 50were apparently healthy as control.

Regarding to coagulation profile mean±SD of PT in case was 14.3±2.25 compare to 13.2±1.29 in control with statistical significant (*p.value* 0.00). mean±SD of PTT in case was 30.1±5.49 compare to 29.7±4.11 in control with no statistical significant (*p.value* 0.71).show in table (4-1). The mean±SD of PT, APTT in age in case group was 14.3±2.25, 30.1±5.5 respectively with no statistical difference *P*.value 0.19, 0.47, respectively. While in male was13.8±2.4, 31.2±7.3 respectively and female was14.6±2.2, 29.39±4.1with no statistical difference *P*.value0.28, 0.26 respectively. In patient with another disease was 14.3±1.94, 30.9±6.75 respectively and for patient with no another disease was 14.4±2.51, 29.3± 4.13 with no statistical difference *P*.value 0.92, 0 .30respectively. In patient intake aspirin was14.4±1.89, 29.9±5.25 respectively and for patient no intake aspirin was 14.1±3.12, 30.6± 6.33 with no statistical difference *p.value* 0 .70, 0 .69respectively. Duration was 14.3±2.25, 30.08±5.49 with no statistical P.value 0.09, 0.57respectively. Show in table (4-2)

Table (4-1): Mean of PT, APTT and INR among study participant (n=100)

Variables	Case group n=50	Control group n=50	*P.Value*
PT	14.3±2.25	13.2±1.29	0.00
APTT	30.1±5.49	29.7±4.11	0.71

Table (4-2): Comparison between Parameters and case Study Variables (n=50)

Variable		PT	APtt	INR	p.value
Age		14.3±2.25	30.1±5.5	1.10±.185	0.19 0.47 0.22
Sex	Male	13.8±2.4	31.2±7.3	1.06±.195	0.28 0.26 0.24
	Female	14.6±2.2	29.39±4.1	1.13±.178	
Another disease	Yes	14.3±1.94	30.9±6.75	1.09±.161	0.92 0.30 0.89
	No	14.4±2.51	29.3± 4.13	1.10±.207	
Aspirin intake	Yes	14.4±1.89	29.9±5.25	1.11±.157	.70 .69 .71
	No	14.1±3.12	30.6± 6.33	1.08±.257	
Duration		14.3±2.25	30.08±5.49	1.102±.185	0.09 0.57 0.12

Chapter five

5. Discussion, Conclusion and Recommendation

5.1 Discussion:

 Hypertension is one of the most common diseases effecting human through the world , because of associated morbidity and mortality and the cost of society ,Evidence for the prothrombotic or hyper coagutable state in hypertension has been extensively reviewed in recent years it has become increasing evident that components of the coagulation and fibrinolytic pathways are primary and secondary predictors of cardiovascular events(Poli *et al* ,2000)

  PT, APTT and INR are significantly higher in case than control with statistical difference *P*.value .00 and .00 for PT and APTT but not with INR Our study revealed no significant difference in pt and Aptt among hypertensive patients compare to normal control group (p.value >0.05); although there no significant difference in pt in correlation to sex and age groups (p.value<0.05), there no significant difference between groups according to duration of HTN (p.value<0.05). .and no significant difference in pt in group of HTN with or without Diabetes Mellitus (DM) (p.value>0.05).the observation point to significant difference among the patient whom under aspirin therapy and those who did not use aspirin (p.value <0.05). The present results shown in this result was consistent to previous study obtained (Sechi et al, 2000). In addition agree to study of (Fathelrahahman, 2007).

5.2 Conclusions

The study demonstrated that hypertension had adverse effects on prothrombin time the measurement of prothrombin and activated partial thromboplastin time where unnecessary when evaluating a hypertensive patients in home where was no clinical evidence of bleeding or of a condition that could produce coagulopathy.

5.3 Recommendations

-further investigation should be done for hypertensive patients, to determine which risk factors and thrombotic markers are important predictors of bleeding and thrombotic risk among hypertensive patients.

-Health education programs about hazards of hypertension disease.

-increase sample size and further studies are needed to elucidate the pathological basis of this observation.

Chapter Six

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Appendixes

Appendixes

Appendix I: Questionnaire

Sudan University of science and technology

College of Graduate studies and scientific research

M.Sc program in Hematology

Evaluation of PT, PTT and INR among Sudanese

Hypertensive patients

Questionnaire No (   )

1-Name: …………………….…………………

2-Age: ………………

3-Gender:

 Male (   )   Female (   )

4-Residence: …………………………………………………………...……

5-Duration of hypertension: ....

6-Any other diseases:

……………………………………………………………………………

7-Aspirin intake: Yes (   )   NO (   )

Investigations:

1). PT: ……………………

2).APTT: ………………..

   Date: /   /

Appendix II

A. Reagents

-PT solution
-APTTsolution

B.Equipment

-Cotton

- Sterile disposable syringe

- Ethanol tabs.

-Gloves

-Centrifuge

-Test tubes

-Micro pipette

-White tips

-Yellow tips

- Tri sodium citrate (TSC 3.2%) container

- Coagulometer

Appendix III: coagulometer