بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduated Studies

Estimation of Antithrombin III Level in Patients with Deep Venous Thrombosis in Khartoum State

قياس مستوى مضاد الثرومبين٣ في المرضى المصابين بتجلط الوريد العميق في ولاية الخرطوم

A dissertation submitted in partial fulfillment of the requirements of M.Sc. degree in medical laboratory science (Hematology and Immunohematology)

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الآية

قال تعالى:

"اللَّهُ نُورُ السَّمَوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ"

صدق الله العظيم

{ سورة النور الآية 35}

Dedication

To the lighting candle in my life and the source of my happiness, my kind mother, my dear father,

My beloved uncle Abdalhag

To that hearts who supply me with eternal flexes and care,

My lovely sisters and brothers,

To all Teachers who learned me, gave me the best of they had, and guided me to the wide knowledge horizons,

To those who shine as stars in my life and stay with me all times, my best friends,

To anyone who help me or encouraged me even by a word when times were hard,

To all people suffering in the universe specially in our third world,

I dedicate this work with much appreciation and sincere wishes for better life full of health and happiness.....

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Abbreviation

Abbreviation	Full text
APC	Activated protein C
APTT	Activated partial thromboplastin time
AT 111	Antithrombin III
DIC	Disseminated intravascular coagulation
DVT	Deep vein thrombosis
FDPs	Fibrin degradation product
HBS	Heparin binding site
HIT	Heparin induced thrombocytopenia
MTHFR	Methylene tetra hydro folate reductase
PAI-1	Plasminogen activator inhibitor
PC	Protein C
PS	Protein S
PT	Prothrombin time
PTS	Post thrombophilic syndrome
RS	Reactive site or Realixe site
TAT	Thrombin Antithrombin III
TPA	Tissue plasminogen activator
TSC	Tri sodium citrate
UFH	Un fractionated heparin
VTE	Vein thromboembolic
VWF	von willebrand factor

Abstract

This is case control study carried at in Bahri teaching hospital, outpatient clinic, Khartoum state, at Sudan University Science and Technology.

The aim of this study was to estimate of Antithrombin III level among DVT patients.

Sixty individual were enrolled, 30 with DVT patients and 30 healthy volunteers were included as control group. Fourteen (46.7%) of patients with DVT were males, while 16(53.3%) of patients were females. Fifteen (50%) of control group were males, while 15 (50%) of control group were females, the selection patients and control group from different Sudanese tribes.

Citrated venous blood sample 2.7 ml was collected with drawn from participant, and using ACCENT 200 Antithrombin III Kit and explore their relation with hypercoagulable state. The results were analyzed by statistical package of social science (SPSS).

The results showed a significant decrease in Antithrombin III level in patients with DVT compared with control group, and Pregnant women also had significantly lower Antithrombin III levels compared to non pregnant women (P. value < 0.05).

There was no statistically significant correlation between Antithrombin III levels an obesity, gender, contraceptive use and age (P. value > 0.05).

In conclusion, Antithrombin III level found to be lower in DVT patients.

مستخلص الأطروحة

هذه دراسة حاله ومجموعة ضابطه اجريت في ولاية الخرطوم في مستشفى بحري,العيادة الخارجيه, جامعة السودان للعلوم والتكنولوجيا.

هدفت الدراسة إلي قياس مستوي مضاد ثرومبين3 في مرضى الجلطات الورديه العميقة.

تم اختيار ستين شخص , 30 منهم مرضى الجلطات الوريديه العميقة بالاضافة إلي 30شخص سليم كمجموعة ضابطه .14(46.7%) من المرضى كانو زكور بينما16 (53.3%) من المرضى كانوإناث 15(50%) من المجموعة الضابطه كانو اناث بينما 15 (50%) من المجموعة الضابطه كانو زكور.

تم اخذ 2.7مل من الدم الوريدي في Tri Sodium Citrate كل مشارك .

واستخدام عدة اكسنت 200مضاد ثرومبين3 ،واكتشاف علاقته بحالة زيارة قدرة التجلط

تم تحليل البيانات احصائيا بواسطة برنامج الحزم الاحصائيه للعلوم الاجتماعية (SPSS).

اظهرت النتائج ان متوسط مستوى مضاد ثرومبين3 كان اقل من وسط مرضى الجلطة الوريديه العميقه بالمقارنة مع المجموعة الضابطة,وكما اظهرت ان هنالك انخفاض ذو دلالة احصائيه عند النساء الحوامل مقارنه مع النساء غير الحوامل (القيمة المعنويه اصغر من 0.05).

كما انه لا توجد دلالة احصائيه بين نقص في مستوى انتي ثرومبين3 ووزن ,ونوع ,واستخدام موانع الحمل وعمر المريض (القيمة المعنويه اكبر من 0.05).

وفي الخاتمه وجد ان مستوى مضاد ثرومبين3 يكون منخفضا عند مرضى الجلطات الوريديه العميقة.

Chapter one

1. Introduction and literature review

1.1 Introduction

Under physiological conditions, the blood remains in a fluid state by maintaining a balance between the procoagulant and the anticoagulant factors present in the blood. When this balance is impaired, a shift towards anticoagulant factors leads to unexpected bleeding and blood loss whereas a shift towards procoagulant factors leads to thrombus formation that occludes blood vessels eventually becoming a cause of stroke. Such undesirable clotting of blood is the most common pathology found in the vasculature. Pathologic clotting of blood, or thrombosis, in the vasculature has been studied extensively since the 18th century (Navaneeth *et al*., 2018).

Thrombosis can occur in arterial or venous vessels and is a complex condition influenced by many factors (Foy and Moll., 2009). Arterial thrombosis occurs rarely in patients with specify Antithrombin III deficiency, because most occlusive events in arteries occur in the setting of underlying arteriosclerosis. It is VTE that is primarily associated with Antithrombin III deficiency (Deitelzweig *et al*., 2011) .Antithrombin III deficiency significantly increases the risk of VTE, typically deep-vein thrombosis in the legs or pulmonary embolism (Di Minno *et al*., 2015) The estimated prevalence of Antithrombin III deficiency varies widely, with estimates between 1:500 and 1:5000.This broad range typifies the difficulties in ascertaining the true prevalence of a relatively uncommon disorder in the general population with different subtypes. The annual incidence of venous thrombotic events in patients with familial

Antithrombin III deficiency is estimated to be 0.87–1.6%. Around 50% of individuals heterozygous for these Antithrombin III defects develop venous thrombosis and present with major venous thromboembolic at a younger age than those with other thrombophilic abnormalities (Baglin .,2000) .

1.2 Literature review

1.2.1 Haemostasis

Maintenance of blood fluidity within the vascular system is an important human physiology process. The term of "Haemostasis" refer to the normal response of the vessel to injury by forming a clot that serves to limit hemorrhage. Thrombosis is pathological clot formation that results when haemostasis is excessively activated in absence of bleeding (Haemostasis in the wrong place). Essential components of fluidity, Haemostasis and thrombosis are the blood flows produced by the cardiac cycle, the vascular endothelium and the blood itself .Under normal physiological conditions there delicate equilibrium (eucoaguliblity) between the pathological states of hypercoagulability and hypercoagulability in the circulating blood. (Slavov *et al*., 2011).

1.2.1.2 Haemostatic Response

1.2.1.2.1 Primary Haemostatic Response

Due to the injury an immediate vasoconstriction of the injured vessel and reflex constriction of adjacent small arteries and arterioles is responsible for an initial slowing of blood flow to the area of injury. When there is widespread damage this vascular reaction prevents exsanguinations. The reduced blood flow allows contact activation of platelets and coagulation factors. The vasoactive amines and thromboxane A2 liberated from platelets, and the fibrinopeptides liberated during fibrin formation, also have vasoconstrictive activity (Hbffbrand *et al;*2006), platelets are anchored to the sub-endothelium by links formed between cellular adhesion molecules /adhesive receptors and adhesive ligands / counter receptors in the blood and connective tissue .once adherent to the sub-endothelium the platelets release

their granule contents and also activate platelet prostaglandin synthesis leading to the formation of thromboxane A 2, and additional platelets which are delivered by the following blood .adhere first to basal layer and then to one another through inter-platelets bridges provided by fibrinogen, forming a clot of aggregated cells (first phase of haemostasis).

1.2.1.2.2 Secondary Haemostatic Response

Platelets aggregation requires activation of platelets by adenosine diphosphate, which is released form platelets strong organelles. Fibrin formation represents the second phase of haemostasis .it is triggered by pro-coagulant factors (e.g. fibrinogen, factor v-VWF) secreted by the platelets, by the tissue factor as a critical component of vascular elements (extrinsic pathway) and by contact activation (intrinsic pathway) of coagulation system. Once factor X a Is formed it converts pro-thrombin to thrombin (common pathway), which induce fibrin strand formation (figure1.1).The platelets membranes provide sites for orderly surface packing of activated coagulation proteins (factor v and viii) that urge them to generate thrombin at high rate. The fibrin mesh binds the platelets together and contributes to their attachment to the vessel defect, mediated by binding the platelets report glycoprotein and by interaction with other adhesive proteins such as thrombospondin, fibronectin and fibronectin .The definitive haemostatic plug that seal off hemorrhagic leak is a platelet-fibrin thrombus. Over helming thrombosis induced by activated haemostasis in the vascular bed outside the region of vascular defect is counteracted by the dilution effects of the following blood, by the antithrombotic capacity of the haemostatic system and by local activation of fibrinolysis. Furthermore fibrinolysis with

clot solution paves the way for wound healing (Rosenfeld and Gralnick., 2009).

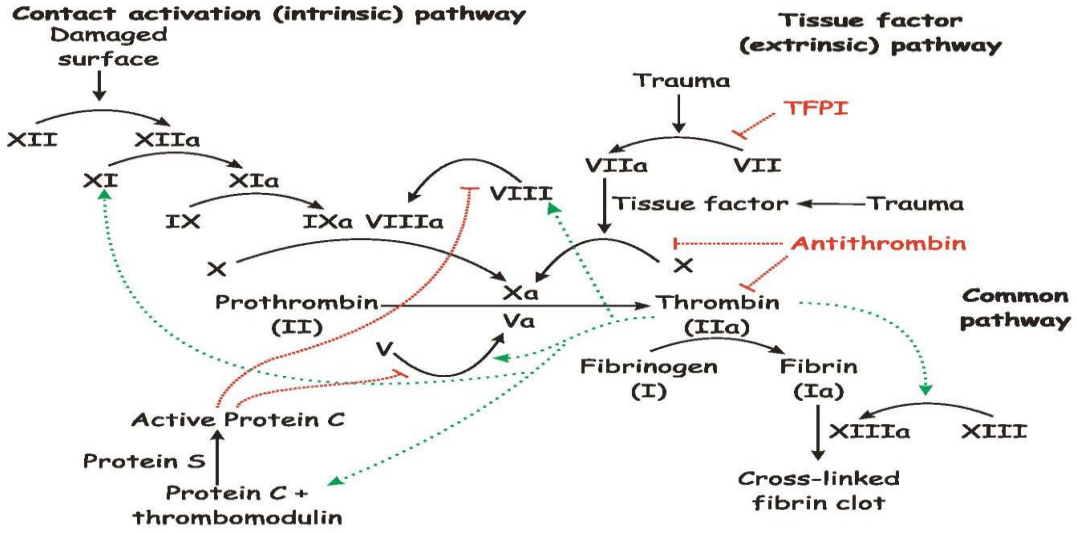

Figure 1.1: Coagulation cascade

Table (1.1): Blood clotting factors

Factor	Name	Pathway
I	Fibrinogen	Both
II	Prothrombin	Both
III	Tissue Factor	Extrinsic
IV	Calcium	Both
V	Proaccelerin	Both
VI	Accelerin	Both
VII	Proconvertin	Extrinsic
VIII	Antihemophiliac	Intrinsic
IX	Christmas Factor	Intrinsic
X	Stuart-Prower Factor	Both
XI	Plasmathromboplastin antecedent (PTA)	Intrinsic
XII	Hageman Factor	Intrinsic
XIII	Protransglutaminase	Both

1.2.1.2.3 Fibrinolysis

A critical link in the chain of hemostasis is the dissolution of fibrin clots, which usually occurs several hours after the stable clot is formed. In this way, blood flow is restored at the local levels and tissue healing is precipitated. The body provides naturally occurring or physiological activators that initiate this process. The key component in this reaction is plasminogen, a plasma enzyme synthesized in the liver with a half-life of 48 hours. Plasminogen is converted to plasmin, chiefly through the action of tissue plasminogen activator (TPA), a substance released through the activity of endothelial cell damage and the production of thrombin. Additional plasminogen activators include factor XIIa, kallikrein, and high-molecular-weight kininogen. Once produced, plasmin, a potent enzyme, does not distinguish between fibrin and fibrinogen and works to digest both to products FSPs/FDPs are formed from plasmin action on fibrin and fibrinogen. As plasmin degrades the fibrinogen molecule, different fragments are split leading to early and late degradation products. Additionally, plasmin also hydrolyzes factors V and VIII, and if circulating in the plasma as pathological free plasmin, the damage to the coagulation system is significant, as clots are dissolved indiscriminately. Of interest is the fact that (TPA) has been synthesized by recombinant technology and is presently used as a pharmaceutical product during stroke episodes for fibrinolytic therapy. As a “clot-busting” drug, it has been effective in thrombotic strokes and if injected within a small time-frame can spare the patient serious stroke side effects. Another plasminogen activator is urokinase, a protease present in the urine and produced by the kidney. The physiological effect of urokinase is minimal in clot dissolution; however, like (TPA) it is a valuable commercial product used in thrombolytic therapy**,**

for patients with heart attacks, strokes, and other thrombotic episodes. Streptokinase is an exogenous fibrinolytic agent, produced when a bacterial cell product forms a complex with plasminogen, a pairing that converts plasminogen to plasmin. This toxic product results from infection with beta-hemolytic streptococci and is a dangerous byproduct if this bacterial strain developed into a systemic infection. It has the most activity on fibrinogen (Betty., 2007).

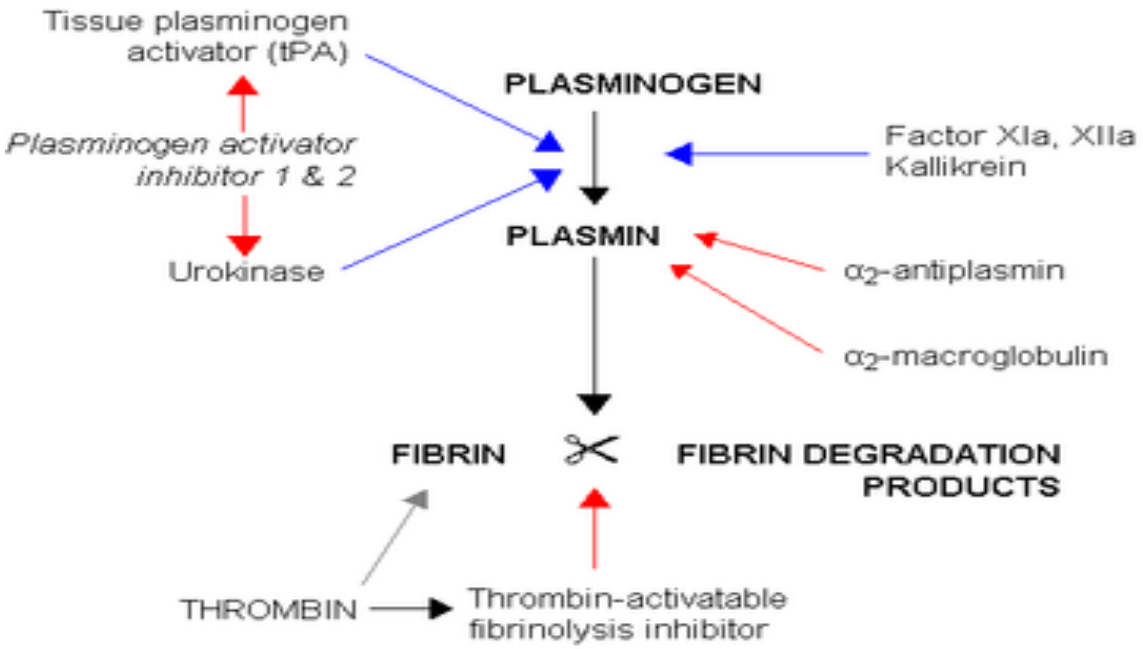

Figure (1.2): Fibrinolytic System

1.2.1.3 Investigation of Haemostasis

The clinical and laboratory approach to the investigation of haemostasis. These tests are generally used as the first step in investigation of an acutely bleeding patient, It should be remembered, however, that these tests examine only a portion of the haemostatic mechanism and have limited sensitivity for the presence of significant bleeding diatheses such as von Willebrand disease (VWD) or disorders of platelets or vessels. Hence a normal 'clotting screen' should not be taken to mean that haemostasis is normal, The investigation properly begins with the bleeding history, which may suggest an acquired or congenital disorder of primary or secondary haemostasis. If the bleeding history or family history is significant, appropriate specific tests and assays should be performed, notwithstanding the results of screening tests such as the PT, APTT and its worth remembering that the tests of coagulation performed in the laboratory are attempts to mimic in vitro processes that normally occur in vivo. The more detailed investigations of coagulation proteins also require caution in their interpretation depending on the type of assay performed like Immunological tests(include immuno-diffusion, immunoelectrophoresis, radioimmunometric assays, latex agglutination (immunoturbidimetric) tests and tests using enzyme-linked immunosorbent assays (ELISA), Coagulation assays, Assays using chromogenic peptide substrates (amidolytic assays)and Other assays include measurement of coagulation factors using snake venoms, assay of ristocetin cofactor and the clot solubility test for factor XIII. DNA analysis is becoming more useful and more prevalent in coagulation. (John *et al* .,2011).

1.2.2 Thrombophilia

1.2.2.1 Introduction of Thrombophilia

Thrombophilia can be defined as a predisposition to form clots inappropriately; Thrombotic events are increasingly recognized as a significant source of mortality and morbidity. The predisposition to form clots can arise from genetic factors, acquired changes in the clotting mechanism, or, more commonly, an interaction between genetic and acquired factors (Khan and Dickerman ., 2006).

In the mid-nineteenth century, the German pathologist Virchow postulated that thrombosis was due to alteration in blood flow (stasis or turbulence), changes in the vessel wall and changes in the composition of the blood (hypercoagulability). In most thrombophilias, impaired neutralization of thrombin or failure to control the generation of thrombin is the cause of thrombosis. Thrombi are composed of fibrin strands and enmeshed blood cells, the relative composition differing between venous and arterial thrombi; Arterial thrombi arise in the setting of high flow and high shear forces in regions where the blood flow is disturbed and in vessels where the wall is damaged. Vessel wall injury leads to platelet activation, which plays a major role in the pathogenesis of arterial thrombi. Venous thrombosis, on the other hand, usually occurs where the degree of vessel wall damage is modest or minimal but where blood flow is abnormally slow and there is local activation of coagulation. Arterial thrombi are composed largely of platelet aggregates and a relatively minor amount of fibrin. Venous thrombi are composed mainly of red blood cells trapped in an extensive fibrin mesh, with relatively few platelets. (Hellstern *et al* ., 2001).

In the last five decades, several genetic risk factors related to thrombophilia have been described. They can roughly be divided into three groups: First group affecting coagulation inhibitors' genes leading to reduced inhibition of coagulation; second group affecting procoagulant factor genes resulting in their impaired inhibition or gain-of-function; Third group affecting fibrinolytic system genes leading to impaired fibrinolysis defects of FV Leiden gene variant, FII G20210A gene variant, FGG C10034T gene variant, PAI-1 4G/5G gene variant, Antithrombin111, PC and PS.

Other genetic risk association between non-O blood group and increased risk of DVT. The concept that thrombophilia could be associated with genetic defects. (Valentina *et al* ., 2014*).*

Additionally dysfibrinogenemia the abnormal production of fibrinogen can result in dysfibrinogenemia. The abnormal fibrinogen usually exhibits an abnormal thrombin-mediated conversion to fibrin. Hyperhomocysteinemia may be both a genetic and acquired abnormality. Hyperhomocysteinemia can be caused by rare inborn errors of metabolism that result in marked elevations of plasma and urine Homocysteine concentrations .They found a marked increase in the risk of venous thrombosis at the highest plasma homocysteine concentrations (Salwa *et al.,* 2006)

1.2.2.2 Epidemiology

Venous thrombosis has an overall annual incidence of < 1 in 1000. It is rare in the pediatric population, with rates of DVT of about 1 in 100,000 and increases in frequency in older patients. While significant advances have been made in understanding congenital thrombophilias, there may still be many more heritable forms of thrombophilia as yet undiscovered. Thus, it is not possible to determine the true prevalence of congenital thrombophilia (Salwa *et al.*, 2006).

1.2.2.3Morphology of Thrombosis

Lines of Zahn **;** these represent pale platelet and fibrin layers alternating with darker red cell–rich layers. Such lines are significant in that they are only found in thrombi that form in flowing blood; their presence can therefore usually distinguish antemortem thrombosis from the bland nonlaminated clots that form in the postmortem state.

Mural thrombi occurring in heart chambers or in the aortic lumen are designated thrombi can develop anywhere in the cardiovascular system. Arterial thrombi are typically relatively rich in platelets, as the processes underlying their development (e.g., endothelial injury) lead to platelet activation. Although usually superimposed on a ruptured atherosclerotic plaque, other vascular injuries (vasculitis, trauma) can also be causal. Venous thrombi **(**phlebothrombosis**)** frequently propagate some distance toward the heart, forming a long cast within the vessel lumen that is prone to give rise to emboli. An increase in the activity of coagulation factors is involved in the genesis of most venous thrombi, with platelet activation playing a secondary role (Vinay *et al*., 2013).

1.2.2.3.1Contrasting arterial and venous thrombosis

Broadly speaking, there are two major types of thrombus:

The white thrombus and the red thrombus .The former is mainly composed of platelets in a mesh of insoluble coagulation proteins such as fibrin, whilst the latter is simply white thrombus plus erythrocytes. However, red clot is the dominant form in venous thrombosis, where white clot is found in arterial thrombosis. Naturally, it is inevitable that there will be some ‘pink’ clot; that is, white clot with a very small number of red cells (Kasthuri *et al*., 2010).

1.2.2.4The thrombophilia screen

1.2.2.4.1 Laboratory tests

When laboratory testing is indicated, it should include assays and tests for heritable defects – deficiency of Antithrombin111, protein C or protein S, FV Leiden and the FII G20210A polymorphism, and also testing for antiphospholipids, the most common acquired cause of thrombophilia. A full blood count, platelet count, APTT, prothrombin time and thrombin clotting time should be incorporated in the initial screening. Liver function tests and measurement of urea and electrolytes are also helpful. The full blood count and platelet count are useful indicators of general health and will identify myeloproliferative conditions that increase thrombotic risk. Factor VIII and homocysteine assays may be considered as 'second line' tests and fibrinogen assays are indicated in patients with prolonged thrombin times. (Baglin *et al*., 2000).

1.2.2.4.2 Other tests

In patients with unexplained clots in the abdomen: JAK-2 mutation, paroxysmal nocturnal hemoglobinuria (PNH) tests. In young patients (<30 years old) with unexplained vein or artery clots: homocysteine. Not to obtain: Methylene Tetra Hydro Folate reductase (MTHFR) genetic test; factor VIII level; (TPA) and PAI-1 blood levels or genetic tests (Ming and Stephan ., 2015).

1.2.3 Deep Vein Thrombosis

Deep vein thrombosis, commonly referred to as "DVT," occurs when a blood clot or thrombus, develops in the large veins of the legs or pelvic area. Some DVTs may cause no pain, whereas others can be quite painful. With prompt diagnosis and treatment, the majority of DVT's are not life threatening. However, a blood clot that forms in the invisible "deep veins" can be life threatening. A clot that forms in the large, deep veins is more likely to break free and travel through the vein. It is then called an embolus. When an embolus travels from the legs or pelvic areas and lodges in a lung artery, the condition is known as a "pulmonary embolism," or PE, a potentially fatal condition if not immediately diagnosed and treated. The symptoms of DVT Approximately one-half of those with a DVT never have recognizable symptoms. The most tenderness in the calf muscles; one may also observe swelling or a change in color of one leg to purple or blue. Acute or sub acute deep vein thrombosis (DVT) usually, but not exclusively, occurs in the legs with an estimated incidence of 1:1000/year(Naess *et al*., 2007) .The most dangerous complication of DVT is pulmonary embolism (PE), which can either be asymptomatic (~30% of DVT patients have silent PE at the time of DVT diagnosis)(Stein *et al*., 2010), or symptomatic (tachycardia, dyspnea, or hypoxemia, which are usually caused by increased right ventricular pressure and/or reduced pulmonary perfusion). DVT and PE are two manifestations of venous thromboembolism (VTE), which may be caused by provoking factors or may occur spontaneously without any of these factors present.(Goldhaber., 2010) Insufficient diagnostic or therapeutic workup for DVT or PE treatment accounts for a considerable number of fatal complications.(Laporte *et al*; 2008) In addition to the acute

mortality, patients surviving with PE are at a considerable risk of developing chronic thromboembolic pulmonary hypertension, which impacts quality of life, treatment costs, and long-term prognosis. Finally, patients with DVT may develop post Thrombotic Syndrome (PTS), which constitutes symptoms and organic changes of the leg veins and tissues caused by increased venous pressure, residual vein occlusion, or venous valve damage following the acute thrombotic event. (Beyer and Schellong.,2005).

1.2.3.2 Epidemyology of DVT

DVT is predominantly a disease of middle age and the elderly. It is very rare before the age of 20years and rare before 40 years. Incidence rates are higher in women especially during childbearing years, Acute or sub acute deep vein thrombosis (DVT) usually, but not exclusively, occurs in the legs with an estimated incidence of 1:1000/year (Naess IA, et al., 2007),the incidence of DVT is reported as 20–35% in western countries 9.6% in Sudan and 12% in Malaysia. Many studies have shown comprehensively that in comparison to whites of European origin, the prevalence of DVT is similar or higher among African-Americans and lower among Asians and Native Americans (Obalum *et al* ;2009).Despite adequate therapy, 1% to 8% of patients in whom pulmonary embolism develops will die (Fedullo *et al*., 2001) .

Term complications such as post phlebitic syndrome (40%) (Pengo V.*et al*; 2004) and chronic thromboembolic pulmonary hypertension (4%) (Kahn *et al*.,2004).

1.2.3.3 Pathogensis of DVT

Venous stasis is the most consequential of the three factors, but stasis alone appears to be insufficient to cause thrombus formation. However, the concurrent presence of venous stasis and vascular injury or hypercoagulability greatly increases the risk for clot formation. The clinical conditions most closely associated with DVT are fundamentally related to the elements of Virchow's Triad; these include surgery or trauma, malignancy, prolonged immobility, pregnancy, congestive heart failure, varicose veins, obesity, advancing age, and a history of DVT. Venous thrombosis tends to occur in areas with decreased or mechanically altered blood flow such as the pockets adjacent to valves in the deep veins of the leg. While valves help to promote blood flow through the venous circulation, they are also potential locations for venous stasis and hypoxia. Multiple postmortem studies have demonstrated the propensity for venous thrombi to form in the sinuses adjacent to venous valves. As blood flow slows, oxygen tension declines with a coincident increase in hematocrit. The hypercoagulable micro-environment that ensues may down regulate certain antithrombotic proteins that are preferentially expressed on venous valves including thrombomodulin and endothelial protein C receptor. In addition to reducing important anticoagulant proteins, hypoxia drives the expression of certain procoagulants. Among these is P-selectin, an adhesion molecule which attracts immunologic cells containing tissue factor to the endothelium. Debate remains regarding the precise location of tissue factor in this process, whether expressed on the endothelium or by cells within the extra vascular tissue, but there is general agreement that tissue factor serves as the primary nidus for thrombus formation. Thrombus formation appears to require both

tissue factor and P-selectin. A venous thrombus has essentially two components, an inner platelet rich white thrombus forming the so-called lines of Zahn surrounded by an outer red cell dense fibrin clot. Fibrin and extracellular DNA complexes with histone proteins forms the outer scaffold, which may be important in determining thrombus susceptibility to tissue plasminogen activator (TPA) and thrombolysis. As the ratio of procoagulants to anticoagulants increases, so does the risk of thrombus formation. The proportion of proteins is in part determined by the ratio of endothelial cell surface to blood volume. A decreased cell surface to blood volume ratio (i.e., large vessels) favors procoagulants. Factor VIII, von Willebrand factor, factor VII and prothrombin seem to be particularly influential in tipping the scale towards coagulation. In addition to promoting thrombin generation, prothrombin inhibits the anticoagulant properties of activated protein C, thereby dampening a natural anticoagulant pathway. There are three such pathways: the protein C anticoagulant pathway, heparin- Antithrombin III pathway, and tissue factor inhibitor pathway. Defects in these pathways are associated with an increased risk for thrombus formation. There are also a number of familial variants that predispose to thrombus formation by increasing the levels of factor VII, VIII, IX, von Willebrand factor, and prothrombin. Other risk factors for clot formation include cancer, oral contraceptives, obesity, and advancing age. Malignancy can exert a compressive effect on veins contributing to stasis. Finally advancing age is associated with an increased risk for thrombosis (Jonathan *et al*., 2017).

1.2.3.4 Classification of DVT

A complex balance of naturally occurring coagulation and fibrinolytic factors, and their inhibitors, serve to maintain blood fluidity and hemostasis. Inherited or acquired changes in this balance predispose to thrombosis.

1.2.3.4.1Inherited predisposition to DVT

The most important inherited biochemical disorders that are associated with VTE result from:

1/Defects in the naturally occurring inhibitors of coagulation deficiencies of Antithrombin III protein C, or protein S.

2/Resistance to activated protein C, which is caused by the factor V Leiden mutation in the majority of cases and A mutation in the 3_ untranslated region of the prothrombin gene (G20210A) (Kearon C, et al., 2000).

1.2.3.4.2 Acquired predisposition to DVT

Acquired hypercoagulable states include estrogen therapy, antiphospholipid antibodies (anticardiolipin antibodies and/or lupus anticoagulants), systemic lupus erythematosus, malignancy, combination chemotherapy, and surgery Patients who develop heparin-induced thrombocytopenia (HIT) also have a very high risk of developing arterial and venous thromboembolism finally hyperhomocysteinemia , whether due to hereditary or acquired causes, is also a risk factor for VTE (Anderson and Spencer.,2003).

1.2.3.5 Types of DVT

1.2.3.5.1 Proximal

When the popliteal vein or thigh veins are involved, symptoms of DVT (pain, swelling, tendermass, and redness) generally do not develop until there is involvement of proximal leg vein. Massive thrombosis can result in vascular compromise and venous gangrene.

1.2.3.5.2 Distal

When the Calf veins are involved. It is usually asymptomatic Clinically, proximal vein thrombosis is of greater importance and is associated with serious chronic diseases such as active cancer, congestive cardiac failure, respiratory insufficiency, or age above 75 years, whereas distal thrombosis is more often associated with risk factors such as recent surgery and immobilization (Emeka *et al*.,2014).

1.2.3.6 Causes of DVT

DVT is generally caused by a combination of two or three underlying conditions .The most common sites of deep vein clots are the lower leg and thigh. They can also occur in the pelvis and arm. Causes of a thrombus (blood clot) include slow blood flow, an injury to the lining of a vein, or having blood with an increased tendency to clot.

1/Limited movement can cause slow blood flow, which increases the risk of DVT. Limited activity can occur with prolonged bed rest after surgery or because of illness.

2/Injury of a deep vein from fracture, surgery, or severe muscle injury can lead to DVT.

3/Type equation here Estrogen in birth control pills or hormone therapy makes blood more likely to clot.

Clotting risk is also higher during pregnancy and for up to 6weeks after giving birth because of increased estrogen (Wells PS *et al*., 2014).

1.2.3.7 Clinical Features of DVT

History and clinical examination are not reliable ways of diagnosing DVT. Lower extremity DVT can be symptomatic or asymptomatic. Patients with lower extremity DVT often do not present with erythema, pain, warmth, swelling, or tenderness. Symptomatic patients with proximal DVT may

present with lower extremity pain, calf tenderness, and lower extremity swelling. Homans' sign may be demonstrable in DVT. Most of these features lack specificity; hence clinical evaluation usually implies the need for further evaluation. The left leg is the commonest site for venous thrombosis in pregnancy and in acute massive venous thrombosis. This may be due to compression of the left iliac vein by the right iliac artery (May–Thurner syndrome).Stasis and endothelial injury are important in DVT following trauma or surgery while hypercoagulability is responsible for most cases of spontaneous DVT(Emeka *et al*., 2011).

1.2.3.8 Diagnosis of DVT

In the event that the patient's history, manifestations, and physical exam propose a DVT, tests are expected to affirm this. Tests to analyze DVT may incorporate pressure ultrasonography, differentiate venography, attractive reverberation imaging (MRI), figured tomography (CT check), or potentially a blood test called D-dimer. In the event that a man with a DVT likewise has signs or side effects of a pneumonic embolus, extra testing will be required (Destefani and Taufner ., 2017).

1.2.3.9 Treatment of DVT

1/Heparin followed by warfarin (Coumadin).

2/Warfarin once acute anticoagulation is achieved warfarin is the drug of choice for long-term therapy to prevent clot recurrence.

3/Un-fractionated Heparin Treatment with un-fractionated heparin is based on body weight and the dosage is titrated based on the APTT.

4/Low-Molecular Weight Heparin compared with un-fractionated heparin.

5/Enoxaparin (Lovenox).

6/Dalteparin (Fragmin), another LMW heparin, is approved only for prophylaxis of DVT (Dino *et al*., 2001).

1.2.3.10 Recurrent of DVT

Patients with a history of venous thrombosis are at increased risk of recurrence irrespective of whether or not they have an identifiable thrombophilia. The risk of recurrent venous thrombosis after stopping oral anticoagulant therapy in subjects heterozygous for FV Leiden has been compared with that in subjects with no detectable thrombophilia. A higher recurrence rate in FV Leiden homozygotes may be at increased risk. Some studies have suggested that recurrent venous thrombosis after discontinuation of warfarin therapy is high in patients with Antithrombin111or protein S deficiency but other studies have failed to corroborate this finding and have suggested that the recurrence rate may be similar to that in unselected patients with venous thrombosis (Seligsohn and Lubestky., 2001).

1.2.3.11 Prophylaxis for DVT

The prevention of DVT remains a problem despite the development of several prevention regimes. This has been due to the lack of essential knowledge on the exact nature of the 'trigger mechanism' which initiates thrombosis in the leg and the absence of sensitive and accurate technique for measuring with precision the effects of prophylaxis. To some extent, the second difficulty has been overcome by using the 125 Iodine – labeled fibrinogen test. It is now possible to determine the true incidence of this disease and the effectiveness of a specific regime of prevention can be judged with greater accuracy. The main attempts to prevent deep vein thrombosis can be categorized under two groups; those directed at eliminating stasis in the deep veins and those directed at counteracting changes in blood coagulability (Obalum *et al*.,2009).

1.2.4 Antithrombin III (AT III)

1.2.4.1 Introduction of Antithrombin III

A member of serpin (serine proteinase inhibitor) super-family is the most important endogenous anticoagulant. Antithrombin III is an a2-globulin synthesized predominantly in the liver, has a half-life of approximately 2-4 days and a molecular weight of 58,000 Dalton, and contains 432 amino acids. There are two isoforms of the Antithrombin III protein in circulation, the α (90–95%), has oligosaccharide chain of almost identical biantennary structure attached to each of its four N-glycosylation consensus sites and β(5–10%) isoforms. The β-isoform shows a higher affinity for heparin due to lack of glycosylation at Asn 135 however, its exact physiological role remains to be ascertained. Antithrombin III, The significance of Antithrombin III in hemostasis is evident by the fact that its heterozygous deficiency is associated with increased risk of thrombosis whereas homozygous deficiency might be fatal. Antithrombin III regulates coagulation by inhibiting thrombin, factors IX, Xa and XI of the blood coagulation system. Antithrombin III has evolved a complex heparin induced conformational change mechanism to efficiently inhibit these proteases. However this has also made Antithrombin III prone to structural and functional defects .The first mutation linked to Antithrombin III deficiency was characterized in 1983. Antithrombin III deficiency may be either type I, where both the activity and antigen levels in the plasma are reduced or type II, where normal antigen levels are associated with a reduced Antithrombin III activity level (Bhakuni *et al*., 2015).

1.2.4.2 Gene/Gene expression of Antithrombin III

The gene encoding human Antithrombin111 gene (*SERPINC1*) is located on chromosome 1q23–25. It has 7 Exons spanning 13.4 kb of DNA (Kenneth *et al*., 2016).

1.2.4.3 Function of Antithrombin III

Antithrombin III (AT, formerly AT-III) is a 58-kD glycoprotein that functions as a potent natural anticoagulant and is estimated to provide 80% of the inhibitory activity against thrombin. Antithrombin III is a serine protease inhibitor (serpin) that inactivates many enzymes in the coagulation cascade, though thrombin and factor Xa are its primary targets. In plasma, there are two isoforms of Antithrombin III: α less potent inhibitor of coagulation , and β, a potent inhibitor enriched in blood vessel walls. In contrast to some direct thrombin inhibitors, which reversibly and transiently block thrombin activity, Antithrombin III inhibition of thrombin is irreversible. Antithrombin III inhibits coagulation enzymes in a slow, progressive manner in the absence of heparin and heparin-like glycosaminoglycans. However, the presence of heparin induces conformational changes in Antithrombin III that result in at least a 1,000-fold enhancement of Antithrombin III activity. In addition to its potent anticoagulant activity, Antithrombin III possesses anti-inflammatory properties, many of which are mediated by its actions in the coagulation cascade. Most importantly, thrombin inhibition by Antithrombin III blocks activation of many inflammatory mediators. Besides blocking thrombin-induced inflammatory pathways, Antithrombin III also inhibits other coagulation enzymes that stimulate the production of several inflammatory Mediators, including interleukin (IL)-6, IL-8, E-selectin, and other molecules involved in monocyte recruitment and adhesion to endothelial

cells. Antithrombin III may also interfere in the formation of complexes between tissue factors and factor VIIa, complexes that promote synthesis of inflammatory cytokines and chemokines (George ., 2009).

1.2.4.4 Mechanism of Action of Antithrombin III

Antithrombin III a natural anticoagulant—is a serine protease inhibitor (SERPIN) that primarily inactivates multiple enzymes generated by the coagulation cascade, including factors IIa (thrombin), Xa, and IXa and, to a lesser extent, factors XIa and XIIa as well as kallikrein and plasmin (Figure 1).(James AH, *et al*.,2013.) Antithrombin III has also been shown to have a subsidiary role to tissue factor pathway inhibitor in the inactivation of factor VIIa-tissue factor. Antithrombin III is synthesized by the liver and also indirectly prevents the activation of protein C by inhibiting thrombin. Activated protein C forms a complex with free protein S in the presence of calcium on the activated platelet surface to inhibit factors VIIIa and Va. The proteolysis of factors VIIIa and Va prevents the activation of factor X and prothrombin, respectively, thereby limiting the generation of thrombin. The mechanism by which Antithrombin III functions primarily involves 2 distinct domains on the molecule: an active reactive center and a heparin-binding site. The arginine-reactive center of Antithrombin III interacts with the active serine site of coagulation proteases. In the absence of heparin or heparin sulfate, Antithrombin III inhibits the serine proteases of the coagulation cascade at a relatively slow rate. When heparin or other heparin-like glycosaminoglycans (e.g. heparin sulfate) bind to the heparin-binding site, Antithrombin III undergoes a conformational change that enhances its inhibitory activity by >1000 fold. This conformational change, forming a ternary bridging complex, increases the rate of interaction between

Antithrombin III and thrombin, factor Xa, factor IXa, and to a lesser extent factor XIa and factor XIIa (Tanaka and Levy ., 2007).

1.2.4.5 Pathophysiology of Antithrombin III

Role of Antithrombin III in coagulation Antithrombin III is a serine protease inhibitor (serpin) that physiologically inactivates thrombin (factor IIa) and factor Xa (FXa) (Fig. 1) and, to a lesser extent, factors IXa, XIa, XIIa, tissue plasminogen activator (TPA), urokinase, trypsin, plasmin and kallikrein. Antithrombin III physiologically circulates in a form that has a low inhibitory activity. The Antithrombin III -mediated inactivation process for coagulation factors requires the binding of a unique sequence- specific pentasaccharide domain of heparin to the heparin-binding domain of Antithrombin III. This interaction induces a conformational change in Antithrombin III, which accelerates the inhibition of FXa. The inhibition of thrombin, in addition, requires heparin to bind to both Antithrombin III and thrombin, to form a ternary bridging complex, so that then thrombin can be inhibited. The proposed sequence of events is that Antithrombin III first interacts with the pentasaccharide domain, and thrombin then binds to a remote domain of heparin, thus becoming suitably oriented for inhibition. This sequence of events produces tightly bound, irreversible thrombin-AT (TAT) complexes, which are then rapidly cleared from the circulation. In vivo, normal Antithrombin III undergoes a slow conversion to a latent form that is not only inactive by itself but also dimerizes with an active Antithrombin III molecule (with preferential binding to the b-isoforms of Antithrombin III). This reaction, which normally has minimal physiological consequences, is accelerated with an increase in the body temperature, explaining episodes of acute thrombosis in families with conformational unstable Antithrombin III (Rouen-VI AT variant) during febrile episodes. In

addition to its anticoagulant role, Antithrombin III has been found to have an important anti-inflammatory effect that occurs in relation to its interaction with the endothelium. By inhibiting thrombin and FXa, it reduces the thrombin/FXa-mediated release of pro inflammatory cytokines such as interleukin 6 and interleukin 8. By binding to heparin sulphate on the endothelium, Antithrombin III increases the production of the important anti-inflammatory cytokine prostacyclin, which then mediates smooth muscle relaxation and vasodilatation and inhibits platelet aggregation. The anti-inflammatory effects of Antithrombin III are closely dependent on its ability to bind with the endothelial glycosaminoglycans and are not observed in its reaction with commercial/free circulating heparin Levels of Antithrombin III associated with development of thrombosis Normal plasma levels of Antithrombin III range from (112 to 140 lg / mL).Types of Antithrombin III deficiency Hereditary Antithrombin III deficiency Inherited Antithrombin III deficiency is divided into type I deficiency , in which both the functional activity and antigenic levels Antithrombin III are proportionately reduced (quantitative deficiency), and type II deficiency, in which normal antigen levels are found in association with low Antithrombin III activity due to a dysfunctional protein (qualitative deficiency). Type II deficiencies can be further sub-classified into three types, depending on the location of the mutations and, consequently, the performance of different Antithrombin III assays (Patnaik and Moll; 2008).

1.2.4.6 Epidomlogy of Antithrombin III

The relative incidence of congenital Antithrombin III deficiency is between 1:2,000 and 1:5,000. Antithrombin III deficiency is inherited as an autosomal dominant disorder. Homozygotes have not been reported in Antithrombin III deficiency. Patients manifest signs and symptoms of

between 10 and 30 years of age, their first thrombotic event. An initial event is spontaneous in approximately half of patients. Women frequently experience manifestations during pregnancy or because of oral contraceptive use. Decreased levels of Antithrombin III usually correlate with the severity of venous thrombosis. Arterial thrombosis is a less common finding in Antithrombin III deficiency (Mary and Turgeon., 2012).

1.2.4.7 Classification of Antithrombin III deficiency

1.2.4.7.1 Heritable Antithrombin III deficiency

Two major phenotypes of heritable Antithrombin III deficiency are recognized. Type I is characterized by a quantitative reduction of qualitatively normal Antithrombin III. Type II deficiency is due to the production of a qualitatively abnormal Antithrombin III protein. In both types of Antithrombin III deficiency, Antithrombin III activity is reduced to a variable extent. In type I deficiency Antithrombin III antigen levels are reduced concordantly with the functional reduction. In type II deficiency, Antithrombin III antigen levels are discordantly higher than the functional levels and may be close to normal (Oven ., 2006).

1.2.4.7.2 Acquired Antithrombin III deficiency

Acquired AT-III deficiency can be caused by decreased synthesis, increased consumption, or other disorders; it can also be drug induced. Acquired deficiency of ATIII can be found in patients who have had liver cirrhosis, liver cancer, nephropathy, disseminated intravascular coagulation (DIC), sepsis, preeclampsia, or trauma, and in patients receiving l-asparaginase, oral contraceptives, severe toxicants, or heparin therapy. Overall, patients with the acquired type of Antithrombin III deficiency are exposed to a high risk of thromboembolism, due to depletion of a protein critical to anticoagulation in plasma. Low Antithrombin III levels could be detected not only during

but also before the thrombotic event. Acquired Antithrombin III deficiency occurs in different medical conditions with a similar risk of thrombosis (Zeyuan *et al*., 2017).

1.2.4.8 Signs and symptoms of Antithrombin III

Clinical presentations of patients with deficiencies of naturally occurring anticoagulants are similar. Deficiencies of 50% of normal for protein C, protein S, and AT-III may lead to serious thrombotic events. Frequent presenting conditions include thrombophlebitis, deep venous thrombosis, and pulmonary emboli (Mary *et al*., 2012).

1.2.4.9 Laboratory Evaluation of Antithrombin III

1/Antigenic Assays the first assays developed for detection of Antithrombin III deficiency quantified the antigenic form of the molecule by radio immunodiffusion techniques or Laurell rocket electrophoresis. Little impetus has existed for the development of modern enzyme immunologic assay methods, and most laboratories still perform commercial radial immunodiffusion methods to quantify the antigenic form of Antithrombin III.

2/Functional Assays almost all current methods measure functional levels of the Antithrombin III protein by use of synthetic substrate technology using predominantly amidolytic methods. Normal range for Antithrombin III activity obtained from normal blood donors was determined to be 83% to 128%.

3/Genetic analysis has been important in identifying the various specific mutations mentioned, but for the diagnostic clinical laboratory and routine medical practice, these data are likely to have little relevance beyond the classification of types I and II.

4/Test Application The issue of who should be tested is relatively easy in the case of a patient with a strong family history of thrombosis or in a young individual with thrombosis and no apparent family history. Also, recognition of the high frequencies of the factor V Leiden and prothrombin G20210A mutations in conjunction with the multiple-hit etiology of thrombophilia means that most patients now will have a panel of the more common thrombophilia markers analyzed during an evaluation (Kandice ., *et al*., 2002).

Many techniques for the analyses of Antithrombin III genetic alterations have been developed previously. The most sensitive mutation detection technique is considered to be direct sequencing; however, the sequencing of Antithrombin III gene is technically demanding, time consuming, and costly. (Li-Ping *et al*., 2010).

1.2.4. 10Some cases patients should not be investigated for Antithrombin III deficiency

1/In patients who are receiving a vitamin K antagonist, Antithrombin III levels will be substantially decreased and this is an acquired (and expected) finding.

2/In patients who are pregnant or taking an oral contraceptive, Antithrombin III levels will be mildly to moderately decrease.

(Lipe *et al*., 2011).

1.2.4.1 1Treatment of Antithrombin III

1/ Antithrombin III concentrates

2/ Heparin therapy is for all patients with Antithrombin III deficiency, should avoid administration of oral contraceptives (Amelia and Gaman ., 2014).

1.2.4.1 2Management of Antithrombin III deficiency

Acute thrombosis can usually be managed with low-molecular-weight heparin (LMWH). Some patients may be resistant and, therefore, require higher doses of LMWH or un-fractionated heparin (UFH). This would be evident, for example, when the activated partial thromboplastin time (aPTT) is not prolonged despite adequate UFH administration. Alternative anticoagulants that are antithrombin-independent (e.g. argatroban, rivaroxaban) may be considered. (Monagle *et al*., 2012).

1.2.5 Previous studies

A study by Bhakuni *et al,* in (2015) in India. Screened Antithrombin III deficiency in Indian Patients with Deep Vein Thrombosis screened 1950 deep vein thrombosis (DVT) patients for Antithrombin III activity and antigen levels 2.66% patients had low Antithrombin III levels. This is the first Indian study where novel and known variants are identified in AT III gene was significant in DVT population (*P*. value> 0.05) (Bhakuni *et al*., 2015).

Another study by Heidelberg *et al* , in (2013) in Berlin. Antithrombin III deficiency is a rare factor of thrombophilia with a mean prevalence of 0.02 % in the general population, associated with a more than ten-fold increased risk of DVT (Heidelberg *et al*., 2013).

A study by Li-Ping *et al,* (2010**)** in China , who found Antithrombin deficiency is one of the risk factors for DVT, the incidence of AT deficiency is approximately 0.02%-0.17% in the general population and 4%-7% in patients with familial thrombosis and the incidence of VTE are 0.1%-0.5% (Li-Ping *et al*., 2010).

Additional study by Van Boven *et al* , in (2018) in Netherlands. Had reported venous thromboembolism is a multicausal disorder. This family study showed a 20-fold increased risk for venous thromboembolism in antithrombin-deficient individuals versus non deficient individuals (van Boven *et al* ., 2018).

A study by Peter *et al* (2015) .Had reported women with Antithrombin III deficiency have an increased risk for pregnancy-associated venous thromboembolic (Peter *et al* .,2015).

1.3 Rationale

Although many causative factors are now known to predispose an individual to thrombotic events may inherited or acquired in proportion of patients with thrombosis.

DVT is a potentially dangerous clinical condition that can lead to preventable morbidity and mortality.

Evaluate Antithrombin III level in Sudanese patients with DVT, because there is a few published data concerning DVT risk factors among Sudanese population like surgery or trauma, malignancy, prolonged immobility, congestive heart failure, varicose veins, obesity, and pregnancy This study will help whether AT deficiency – which is a known risk factors for venous thrombosis is contributes to the incidence of DVT among Sudanese or not

By contribute, the findings of this study could contribute an important role in decreasing morbidity and mortality of DVT patients. Furthermore, this study could be used as a reference or a benchmark study for related studies.

1.4 Objectives

1.4.1 General Objective

To evaluate Antithrombin III level in Sudanese patients with DVT.

1.4.2 Specific Objectives

1. To estimate Antithrombin III level in patients with DVT using immunological assay.
2. To correlate age of incidence in patients with DVT with Antithrombin III level.
3. To compare Antithrombin III level according to patients gender, and known DVT risk factors trauma, malignancy, prolonged immobility, congestive heart failure, varicose veins, obesity, and pregnancy
4. To evaluate Antithrombin III level in pregnant women.

Chapter Two

2 Materials and Methods

2.1 Materials

2.1.1 Study design

This study was case control study, conducted in the period between April-July 2018at Sudan University Science and Technology.

2.1.2 Study area

The study was performed in Khartoum state.

2.1.3 Study population

The study was carried out on patients admitted to Bahri hospital from outpatient unite and health centers in certain areas in Khartoum state from different age groups and both genders.

2.1.4 Inclusion criteria

Sudanese patient with DVT and pregnancy were included.

2.1.5 Exclusion criteria

Patients on anticoagulant therapy and vitamin K were excluded.

2.1.6 Data collection

Data was collected using designed patients questionnaire to obtain demographic and clinical.

2.1.7 Sample Size and Speciment

Sampling method was a simple random sampling technique. The sample size was 60, 30 patients with DVT and 30 apparently healthy controls.

2.1.8 Ethical considerations

Ethical committee of research in the Faculty of Medical Laboratory Science was approved the study. The purpose and objectives of the study was explained to each one of participants, the participant has right to voluntary informed consent, has right to withdraw at any time without any deprivation, assured them that the data collected will remain confidential and it's not allowed for any person to identify it. The questionnaire was filled in their rest time, and participant has right to benefit from the researcher knowledge and skills. Samples were coded and confidentiality of patient data was maintained throughout the study by locking hard copies and password protecting electronic files.

2.2 Methodology

2.2.1 Speciment collection

2.7mlcitrated venous blood sample was collected from individual under study and dispensed in Tir Sodium Citrate (TSC) container for measurement of Antithrombin111 by using ACCENT 200 Antithrombin111 Kit.

2.2.2 Measurement of Antithrombin III

Antithrombin III level was measured by immunoassay using automated mindray BS-200 machine and ACCENT 200 Antithrombin111 Kit.

2.2.3 Method Principle

The Antithrombin III presents in a sample form with the specific Antibody an Immunological complex. The increase of turbidity after the addition of antiserum measured at ƛ= 340nm is proportional to Antithrombin III concentration in the sample.

2.3 Quality Control

For internal quality control it was used the Comay immuno-control III (Cat. No 4-291) with each batch of samples. For the calibration of automatic analyzers systems the Comay immuno-multical (Cat. No 4-287) is recommended. As a 0 calibrator 0.9% NaCl should be used. The calibration curve should be prepared every 4 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range (ACCENT-200 Antithrombin III).

2.4 Data analysis

The data obtained from the automated mindray BS-200 machine and collected by questioner was entered and analyzed by using Statistical Package for Social Science (SPSS) version 23 (SPSS INC, Chicago, IL, USA). The mean and standard deviation were used to summarize Antithrombin III level. Independent T test (analysis of variance) was done to compare the mean Antithrombin III difference across the two groups. Pearson correlation test was used to investigate correlation between continuous variables, P. *value* of <0.05 was considered as statistically significant. Tables and figures were used for the description of the data.

Chapter Three

3. Results

3.1 Demographic Data

A total of 60 subjects were enrolled in this study; 30 of them Sudanese patients diagnosed with DVT and 30 age matched apparently healthy volunteers as a control group; 14(46.7%) of patients were males and 16(53.3) were females, 4(13%) from women were pregnancy; while 15 (50%) of the control group were males and 15(50%) were females (Figure 3.1).

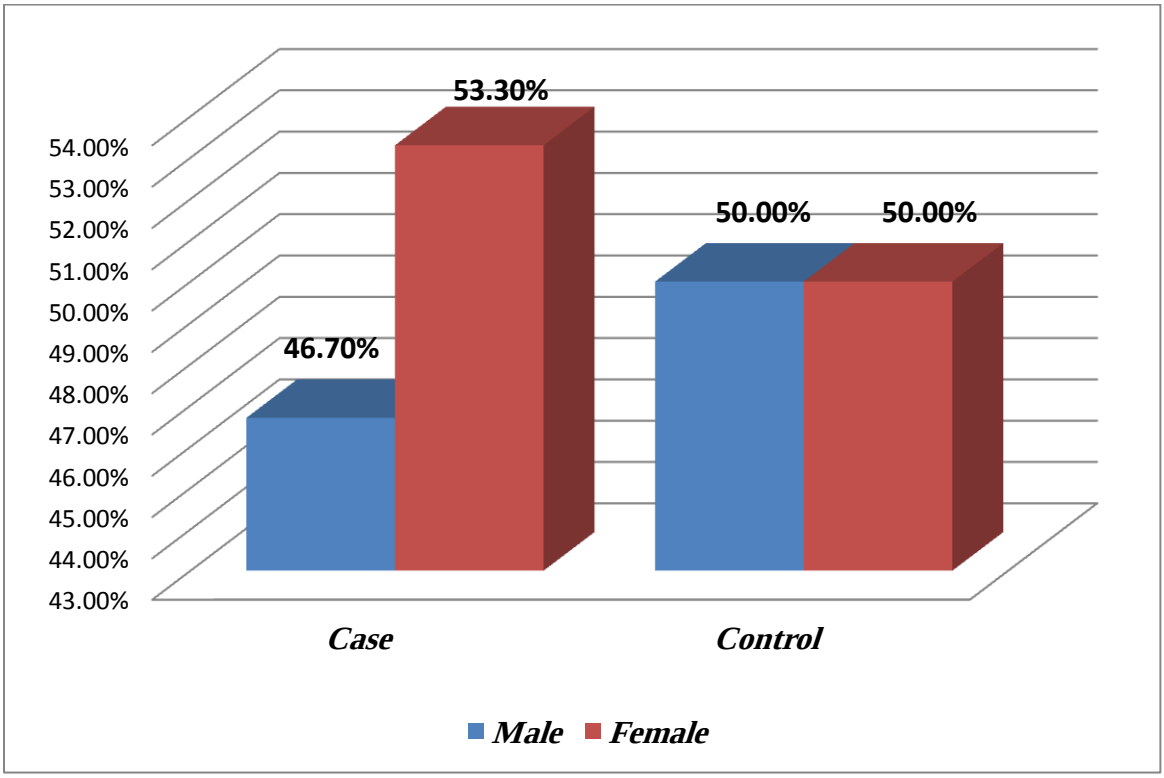

Figure (3.1) Gender Distribution among study subjects

Age of patients ranged from 19 to 72 years (Mean ±SD=41.77 ±14.12) and age of the control group ranged between 18 and 68 years (Mean ±SD= 35.33±10.49) (Table3.1).

Table (3.1): Comparison of age in patients and control groups

Group	Mean	SD	*P. value*
Case	41.77	14.12	0.058
Control	35.33	10.49	

Nine (30%) of the patients were using contraceptives, 9(30%) were obese (BMI>31), and 4(13.3%) were pregnant women.

Thirteen (41.4%) of the patients aged<40 years whereas 17(58.6%) of them were >40 years (Table 3.2).

Table (3.2): Base line characteristic of patients

Variables	Frequency	Percentage (%)
Contraceptive		
Yes	9	30.0
No	21	70.0
Obesity		
Yes	9	30.0
No	21	70.0
Pregnancy		
Yes	4	13.3
No	26	86.7
Age		
≤40 Years	13	41.4
> 40 years	17	58.6
Total	**30**	**100.0**

The mean concentration of Antithrombin III level was lower in patients compared to control group, and the difference was statistically significant (Table 3.3).

Table (3.3): Mean level of Antithrombin111 in case verses control group

Parameters	**Group**	**Mean(mg/l)**	**SD**	***P. value***
AT111level	Case	30.07	7.71	0.005
	Control	35.83	7.88	

There was significant decrease in mean Antithrombin III level when compared in women using contraceptives and those not using contraceptives in control group (Table 3.4).

Table (3.4): Mean concentration of Antithrombin111 due to contraceptives were used in DVT patients with control group

AT 111	**Mean**	***SD***	***P. value***
Contraceptive	13.38	0.495	0.005
Control	31.467	7.239	

Individual with BMI >31 were considered obese; obesity was found to have not effect on Antithrombin III level (Table 3.5).

Table (3.5): Comparison of mean concentration of Antithrombin III according to obesity in DVT patients

Obesity	Mean	SD	*P. value*
Yes	27.84	8.77	0.310
No	31.01	7.24	

The mean concentration of Antithrombin111 was significantly decrease in pregnant women compared to control group (Table 3.6).

Table (3.6): Comparison of mean concentration of Antithrombin III in pregnancy woman in DVT with control group

ATIII	Mean	SD	*P. value*
Pregnancy	17.21	0.419	0.001
Control	31.12	8.424	

Although, Mean Antithrombin111level was lower in females than males, but the difference was not statistically significant (Table3.8).

Table (3.7): Comparison of mean concentration of Antithrombin III according to patients gender

Gender	Mean	SD	*P. value*
Male	32.42	7.42	0.119
Female	28.01	7.59	

There was no statistically significant difference in mean Antithrombin III level when compared in patients aged<40 years and those aged more than 40 years (table3.9).

Table (3.8): Comparison of mean Antithrombin III level according to Age group of DVT patients

Age	Mean	SD	*P. value*
≤40 Years	28.70	7.71	0.094
> 40 years	31.80	7.17	

Also no statistically significant correlation was found between Antithrombin III level and patients age (Figure 3.2)

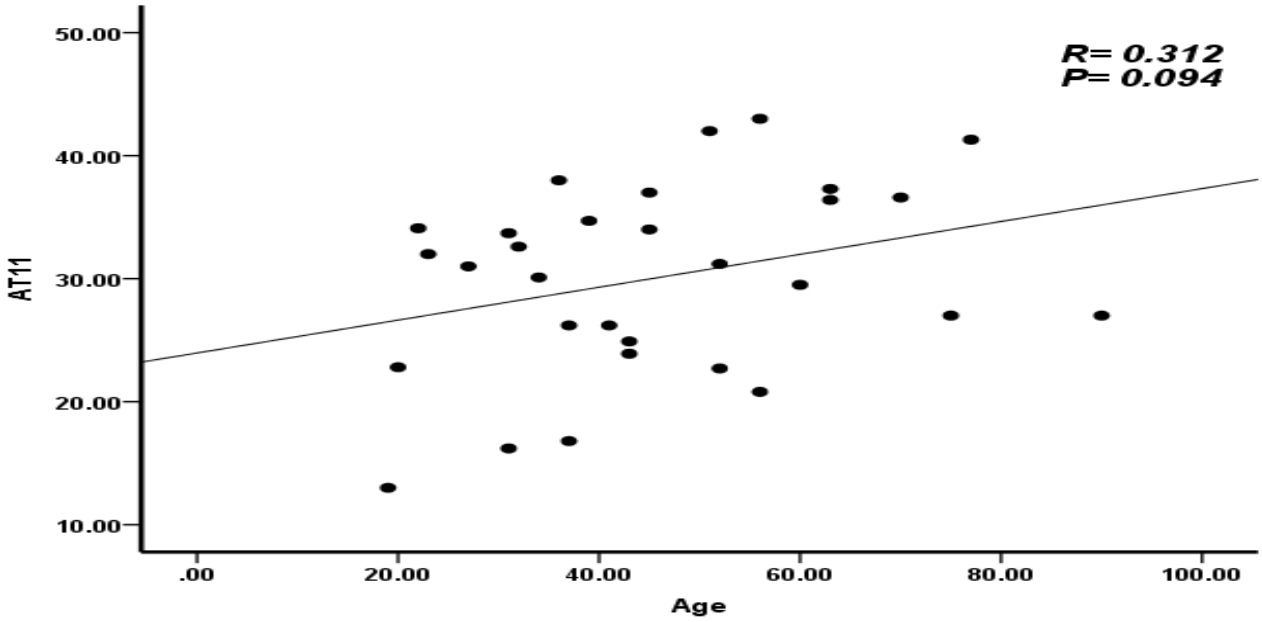

Figure 3.2 Correlation between Antithrombin III level and patients age

Chapter Four

4 Discussion, Conclusions and Recommendations

4.1 Discussion

This study showed that the mean Antithrombin III level in DVT patients was significantly decrease when compared to control group.

These findings agreement with that of study done Bhakuni *et al*, (2015) in India, who reported patients with DVT had low Antithrombin III level (Bhakuni, *et al*., 2015).

And also these result agreement with Li-Ping *et al*, (2010**)** in China , who found Antithrombin III deficiency is one of the hereditary risk factors for DVT, the incidence of AT deficiency is approximately 0.02%-0.17% in the general population and 4%-7% in patients with familial thrombosis and the incidence of VTE are 0.1%-0.5% (Li-Ping *et al* ., 2010).

Additionally these result agreement with Van Boven *et al* (2018) in Netherlands, who finding venous thromboembolism is a multicausal disorder. This family study showed a 20-fold increased risk for venous thromboembolism in antithrombin-deficient individuals versus non deficient individuals (van Boven *et al* .,2018).

In the present study the Antithrombin III level had lower significant by administration of oral contraceptive. This result agreement with Petersen *et al* (2018), in Wichita, who reported depression of antithrombin III in women receiving oral contraceptives" and in postpartum women as much as a week after delivery. This depression has been implicated in the increased incidence of thrombotic episodes in these women (Petersen *et al* .,2018).

The result of the present study showed that, statistically significant relationship between Antithrombin III deficiency and pregnant women that present study determined the mean of Antithrombin III level in pregnant women were decreased. This result agreement with Nwogoh *et al*, (2016) study in Benin City, he reported that, the mean of Antithrombin III level in pregnant women is within normal range in pregnancy but there is a significant decline in level in the third trimester(Nwogoh, *et al.*,2016).

This result of the present study also agreement with Andra *et al* (2014), who found that overall Antithrombin III levels were 20% lower than baseline during pregnancy (Andra *et al* .,2014).

This result of the present study also agreement with Peter *et al* (2015), who found women with Antithrombin III deficiency have an increased risk for pregnancy-associated venous thromboembolic (VTE) (Peter *et al* .,2015).

Regarding the effect of pregnant women on Antithrombin III level disagree with Mirjana K et al, (2011**)** in Belgrade-Serbia, who finding Investigation of hemostatic changes during pregnancy, especially natural coagulation inhibitors, showed no significant change of Antithrombin III level, considering gestation age by healthy women without prior complications during pregnancy and without history of thrombosis(Mirjana K *et al.*, 2011).

Regarding the effect of obesity on Antithrombin III level disagree with Ayman *et al* (2017), who finding Obesity as a substantial component of metabolic syndrome may also have elevated plasma levels of Plasminogen activator inhibitor-1(PAI-1) and fibrinogen and decreased protein C levels.

Furthermore, the prothrombotic features of morbidly obese patients include antithrombin III deficiency (Ayman E *et al.*, 2017).

Regarding the effect of age and gender on Antithrombin III disagree with Huma *et al* (2012), in Pakistan, he reported that earlier younger women have

significantly lower concentrations of antithrombin compared with men of the same age, and also reported that the occurrence of menopause in women was accompanied with a significant increase in Antithrombin III levels than their male contemporaries (Huma *et al.*, 2012).

Also regarding the effect of gender agree with Suriana et al, (2017), in Malaysia, Antithrombin III deficiency is a rare autosomal dominant inheritance, affecting men and women equally (Wan *et al* ., 2017).

And also regarding the effect of gender agree with Vincent *et al* (2018), in Nigeria, who found male to female ratio was approximately 1:1(Vincent *et al* ., 2018).

4.2Conclusion

- Antithrombin III level was significantly lower in patients with DVT compared to control group.
- Pregnant women had significantly lower Antithrombin III levels compared to non pregnant women.
- Antithrombin111 deficiency in pregnant women associated with high risk to acquire DVT.
- Antithrombin III level had significantly lower in women were using contraceptives compared to non using contraceptives.
- There was no statistically significant correlation between Antithrombin III levels an obesity, gender, and ages.

4.3 Recommendations

1- Estimation of Antithrombin III level should implemented into the investigation of patients with DVT.

2- Further study should be conducted in the future to screen other thrombophilias in patients with DVT.

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Appendices

Appendix- I Participant information sheet

Principal Investigator: Aya Abdalrhman Mohmed Nasir

Sudan University Sciences and Technology

Introduction: You are being asked to take part in research study on Antithrombin111 in DVT. The study has been approved by Sudan University Sciences and Technology department research ethics committee.

Purpose: The purpose of this study is to evaluate the level of AT111 in DVT patients compared to normal individual.

Risks associated with the study: There is no risk and serious invasive procedure at the beginning as well as at the end of the study and there is no additional time required from you to stay during study.

Benefits of the study: There is no any financial benefit to you. But the result of the study will be used for your clinical care as well as plays a role in diagnosis and will play a role in minimizing mortality and morbidity rate. There is no compensation for using your blood sample.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later on change your mind. Your withdrawal of consent will not affect your right to receive medication. . Also you have the right to have question about the study. I will be glad to answer your questions about this study at any time.

You may contact me at e-mail address Ayaaabdalrahaman@gmail.com.

Appendix- II Questionnaire and Informed Consent Form

Sudan University Sciences and Technology

Graduate College Faculty of Medical Laboratory Science-Hematology

Assessment for AT111 level in DVT patients

Questionnaire

Name…… ID code……

Gender:…… Age:…….

Medication of the patients ? .....

History of thrombosis ?

History of recurrent thrombosis ?

History of DVT family ?

Treatment used ? .....

Other diseases ? .....

Patients have been using cortisol or IF? …….

Informed Consent:

انا المذكور اعلاه اوافق علي المشاركة في البحث المذكور اعلاه لغرض البحث العلمي فقط بمنح عينه دم من الوريد ولي كل الحق بالانسحاب من الدراسة متى ما شئت ولي الحق ايضا في الحصول علي النتائج الخاصه بي والايتم استخدام هذه العينات لاغراض التجارب الانسانيه او الحيوانيه

الامضاء……

- **Precision**

Repeatability (run to run) n = 10	Mean [mg/dl]	SD	CV [%]
level 1	19.4	0.2	1.1
level 2	31.7	0.5	1.7

Reproducibility (day to day) n = 10	Mean [mg/dl]	SD	CV [%]
level 1	19.5	0.4	2.2
level 2	31.4	0.8	2.5

WASTE MANAGEMENT
Please refer to local legal requirements.

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