Sudan University of Science and Technology

College of Post Graduated Studies

Association of Antithrombin III Level , Platelate Count and Platelates Indices among Sudanese Hypertensive Pregnant Women in Khartoum state .

إرتباط مستوى مضاد الثرومبين٣ و عدد و مؤشرات الصفائح الدموية لدى النساء الحوامل السودانيات المصابات بإرتفاع ضغط الدم في ولاية الخرطوم .

A dissertation Submitted for Partial Fulfillment of the Requirements for MSc degree in Medical Laboratory Science (Hematology and Immunohematology).

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بسم الله الرحمن الرحيم

الآية

﴿ هُوَ الَّذِي جَعَلَ الشَّمْسَ ضِيَاءً وَالْقَمَرَ نُورًا وَقَدَّرَهُ مَنَازِلَ لِتَعْلَمُوا عَدَدَ السِّنِينَ وَالْحِسَابَ مَا خَلَقَ اللَّهُ ذَلِكَ إِلَّا بِالْحَقِّ يُفَصِّلُ الْآيَاتِ لِقَوْمٍ يَعْلَمُونَ ﴾

صدق الله العظيم

سورة يونس الآية 5

Dedication

To my parents ,

The person of what I become today ,

Thanks for your great support and continous care .

To my sisters and Brother ,

I am really gratefull to all of you ,

You have been my imperation , and my soul mate .

To all Teachers who learned me,

gave me the best of they had, and guided me to the wide knowledge horizons.

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

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*First of all, my thank to **Allah** for providing me strength and patience until finishing this work.*

*Faithfully, I express my thanks and gratitude to my supervisor **Dr. Nadia Madani** for assistance and orientations that let to completion of this study. My thank is extending to all members of **Hematology and Immunohematolgy Department**, College of Medical Laboratory Science, Sudan University of Science and Technology.*

*Special thanks for **my family** for their financial support beside their precepts and continuous encouragement.*

Abstract

This was analytical case controle study conducted in Khartoum state during the period of January to Octoper 2019 aimed to detect Antithrombin III level deficiency and determine Platelet count and platelet indices in hypertention pregnant women .

A total of 80 subject enrolled in this study , 40 of them worked as cases (pregnant women with hypertention) and the other 60 worked as controls (Normal pregnant women), Control pregnant women were matched to cases in age group .

Venous blood samples (2.5 ml) were collected from the participants in EDTA anticoagulant blood container and 2.7ml of venous blood in Tir Sodium Citrate container blood (TSC). All samples were tested using automated hematology analyzer (sysmex XP-300) for measurement of PLTs count and PLTs indices and Accent 200 Antithrombin III kit for estimation of antithrombin level .

Data were analyzed by statistical package for social science (SPSS) computer program version 20 ,this study pregnant women aged from 18 to 42 .

In conclusion : This study concluded that the mean and standard deviation of Antithrombin III level of induced hypertention pregnant women 0.22±0.03 mg / dl , While , mean ± SD of ATIII level of normal pregnant mg / dl . A high significant decrease of AT III level in women 0.32±0.06 pregnant women induced hypertention in compare with AT III level in normal pregnant women (P. value = 0.00). Mean ± SD of PLT Count of induced hypertention pregnant women 188±41.9 (109L) , While , mean ±

SD of PLT Count of normal pregnant women 233±39.1 (109L), A high significant decrease of PLT Count of induced hypertention pregnant women in compare with PLT Count of normal pregnant women (P. value = 0.00). Mean ± SD of MPV of induced hypertention pregnant women 11.37±1.53 (fl) , While , mean ± SD of MPV of normal pregnant women 10.45±1.58 (fl). Asignificant increased of MPV of induced hypertention pregnant women in compare with MPV of normal pregnant women (P. value = 0.01). Mean ± SD of PDW of induced hypertention pregnant women 16.25±1.86 (fl) , While , mean ± SD of PDW of normal pregnant women 15.29±2.04 (fl)), there is a significant increased of PDW of induced hypertention pregnant women in compare with PDW Count of normal pregnant women (P. value = 0.030). Mean ± SD of PCT of induced hypertention pregnant women 0.21±0.05 (%), While , mean ± SD of PCT of normal pregnant women 0.24±0.06 , there is a significant decrease of PCT of induced hypertention pregnant women in compare with PCT of normal pregnant women (P. value = 0.015).

There was no correlation between AT III level , PLT Count , MPV , PDW and PCT with age and contraceptive.

There was moderate positive correlation between AT III and PLT count , a week negative correlation between AT III and MPV , week negative correlation between AT III and PDW ,a week positive correlation Between AT III and PCT.

deficiency patients were detected by specific Antibody an Immunological complex protocol. Full absent of Antithrombin111 deficiency not dedected . Have been detected neither Antithrombin111 level nor Antithrombin111 activity.

مستخلص الدراسة

هذه دراسة تحليلية تم اجراؤها في ولاية الخرطوم في الفترة ما بين يناير لإكتوبر من عام ٢٠١٩ .

هدفت هذه الدراسة لاكتشاف وجود نقص في مستوى مضاد الثرومبين III وتحديد عدد الصفائح الدموية ومؤشرات الصفائح الدموبة وسط النساء الحوامل المصابات بارتفاع ضغط الدم .

مجموع ٨٠ فرد متضمنين في هذه الدراسة , ٤٠منهم عملن كحالات دراسة (نساء حوامل مصابات بإرتفاع ضغط الدم) وال ٤٠ الاخريات عملن كحالات قياس (نساء حوامل طبيعيات) ، النساء الحوامل حالات القياس تمت مطابقتهم لحالات القياس في مجموعة الأعمار .

عينات الدم الوريدي (2.5 ملي) جمعت من المشاركات في وعاء مضاد التخثر EDTA و(2.7ملي) من الدم الوريدي في وعاء الدم سترات ثلاثي الصوديوم TSC . كل العينات تم إختبارها بواسطة محلل الدم الآلي (سيسميكس اكس بي - 300) لقياس عدد الصفائح الدموية و مؤشرات الصفائح الدموية ، ACCENT 200 لقياس مستوى مضاد الثرومبين .

البيانات تم تحليلها بواسطة تطبيق الحاسب الآلي برنامج الحزم الاحصائية للعلوم الاجتماعية اصداره ٢٠ ، أعمار النساء الحوامل لهذه الدراسة من ١٨-٤٢ سنة .

الإستنتاج : تم إستنتاج الأتي من الدراسة : متوسط النتائج ± الانحراف المعياري لمعدل مضاد الثرومبين III للنساء الحوامل المصابات بارتفاع ضغط الدم 0.03±0.22 ملجرام / ديسيليتر، **و**متوسط النتائج ± الانحراف المعياري لمعدل مضاد الثرومبين III للنساء ذوات الحمل طبيعي 0.06±0.32 ملجرام / ديسيليتر وأيضا وجد نقصان واضح لمعدل مضاد الثرومبين III في دم النساء الحوامل المصابات بارتفاع ضغط الدم عند مقارنته بمعدل مضاد الثرومبين III في دم النساء ذوات الحمل الطبيعي (القيمة المعنوية 0.00).

متوسط النتائج ± الانحراف المعياري لعدد الصفائح الدموية عند النساء الحوامل المصابات بارتفاع ضغط الدم (109) 41.9±188 ليتر ، **و**متوسط النتائج ± الانحراف المعياري لعدد الصفائح الدموية للنساء ذوات الحمل طبيعي (109) 39.1±233 ليتر ، وجد نقصان واضح لعدد الصفائح الدموية عند النساء الحوامل المصابات بارتفاع ضغط الدم عند مقارنتها بعدد الصفائح الدموية للنساء ذوات الحمل طبيعي (القيمة المعنوية 0.00) .

متوسط النتائج ± الانحراف المعياري لمتوسط حجم الصفائح الدموية عند النساء الحوامل المصابات بارتفاع ضغط الدم 1.53±11.37 فيمتوليتر ، **و**متوسط النتائج ± الانحراف المعياري

لمتوسط حجم الصفائح الدموية للنساء ذوات الحمل طبيعي 1.58±10.45 فيمتوليتر، وجدت زيادة في متوسط حجم الصفائح الدموية عند النساء الحوامل المصابات بارتفاع ضغط الدم عند مقارنتها بعدد الصفائح الدموية للنساء ذوات الحمل طبيعي (القيمة المعنوية 0.010) .

متوسط النتائج ± الانحراف المعياري عرض توزيع الصفائح الدموية عند النساء الحوامل المصابات بارتفاع ضغط الدم 1.86±16.25 فيمتوليتر ، **و**متوسط النتائج ± الانحراف المعياري لعرض توزيع الصفائح الدموية للنساء ذوات الحمل طبيعي 2.04±15.29 فيمتوليتر، وجدت زيادة في عرض توزيع الصفائح الدموية عند النساء الحوامل المصابات بارتفاع ضغط الدم عند مقارنتها بعدد الصفائح الدموية للنساء ذوات الحمل طبيعي (القيمة المعنوية 0.030)

متوسط النتائج ± الانحراف المعياري لل PCT عند النساء الحوامل المصابات بارتفاع ضغط الدم (%) 0.05±0.21 ، **و**متوسط النتائج ± الانحراف المعياري لل PCT للنساء ذوات الحمل طبيعي 0.06±0.24 (%) ، وجد نقصان في الPCT عند النساء الحوامل المصابات بارتفاع ضغط الدم عند مقارنتها بعدد الصفائح الدموية للنساء ذوات الحمل طبيعي (القيمة المعنوية 0.015) .

لا توجد علاقة بين كل من مضاد الثرومبين III ، لعدد الصفائح الدموية ، لمتوسط حجم الصفائح الدموية ، عرض توزيع الصفائح الدموية و الPCT مع العمر أو إستخدام موانع الحمل .

توجد علاقة إيجابية متوسطة بين مضاد الثرومبين III وعدد الصفائح الدموية ، علاقة سلبية ضعيفة بين مضاد الثرومبين III و ال MPV ، علاقة سلبية ضعيفة بين مضاد الثرومبين III وال PDW ، وعلاقة إيجابية ضعيفة بين مضاد الثرومبين III وال PCT .

نقص مضاد الثرومبين III اكتشف بواسطه جسم مضاد خاص وذلك باستخدام بروتوكول مناعي معقد غياب كلي لنقص مضاد الثرومبين III غير مكتشف ؛ليس في مستوى او نشاط الانتي ثرومبين III .

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List of Abbreviations

PLT platelet

AT Antithrombin

MCV mean corpuscular volume

MCHC mean corpuscular haemoglobin concentration

APTT activated partial thromboplastin time

PT prothrombin time

TT thrombin time

PIH Pregnancy Induced hypertension

BP Blood pressure

PE Preeclampsia

MKs megakaryocytes

CBC Complete blood count

PIs platelet indices

PCT plateletcrit

MPV mean platelet volume

PDW platelet distribution width

EDTA Ethylenediaminetetra-acetate

TSC Tri Sodium Citrate

DC direct current

SPSS Statistical Package for Soc ial Scienc

CHAPTER I

INTR0DUCTION AND LITERATURE REVIEW

1.1 .Introduction

In pregnancy several haematological , biochemical and hemodynamic modifications occur as part of the physiological adaptation of the body to this condition . These adaptations are meant to maintain the maternal , fetal , uteroplacental homestasis and to prevent excessive bleeding at child birth **(Bonnar J.1987).**

Pregnancy is associated with several changes in platelet count and indices arising from increased platelet consumption in the uteroplacental circulation and haemodilution **(Lyalla *et al* .,2015).**

Platelet indices are potentially useful markers for the early diagnosis of thromboembolic diseases.

AT III is a glycoprotein synthesized in the liver , with a molecular weight of 58,000 and a plasma half-life of about 67 hour. It inhibits action of thrombin and other activated coagulation factors namely Xa IXa XIa , XIIa , Plasmin and Kallibrin.

Hypertensive disorders are most common medical complications of pregnancy, and are one of the major causes of maternal and fetal morbidity and mortality .Thrombocytopenia Complicating hypertensive disorders of pregnancy are responsible for approximately 20% Of all cases of thrombocytopenia during pregnancy **(Tejeswini *et al*.,2016).**

The studies about pregnant women induced hypertention found to be with low platelet count **(Zamir Damani *et al .,2016)*** . While mean platelet volume (MPV) and platelet distribution width (PDW) were increasing as pregnant induced hypertention **(Ahmed *et al* .,2015) .**

Antithrombin III levels were significantly lower in patient with pregnancy –induced hypertention **(Sarkar et al .,2013) .**

In Sudan , where pre-eclampsia and eclampsia are among the main causes of obstetric complication, there is an extremely high maternal mortality **(Ali and Adam, 2011).** This

high frequency of maternal morbidities and mortality needs improvement in obstetric care **(Ali *et al*., 2012).**

Several aspects of preeclampsia in Sudan were investigated **(Elhaj *et al*., 2015)**; however, there were no published research (to the best of our knowledge) on measurement of platelet count and indices in pre-eclamptic patients that carried out to find the role of platelet and platelet indices as predictors of its severity.

1.2 Rationale

Hypertensive disorders are most common medical complications of pregnancy, and are one of the major causes of maternal and fetal morbidity and mortality.
Thrombocytopenia complicating hypertensive disorders of pregnancy are responsible for approximately 20% of all cases of thrombocytopenia during pregnancy .

Preeclampsia is a pathophysiological disorder specific to pregnancy and characterized by vasoconstriction and hypercoagulability **(Matsuda *et al* .,1995) .** In women with preeclampsia, intravascular coagulation frequently coexists leading to thrombus formation in the uteroplacental unit and other maternal organs and thrombocytopenia or hemoconcentration are frequently associated with low AT III activity in pathological conditions. The balance between coagulation and anticoagulation systems is shifted towards coagulation, and thus there is a thrombotic tendency **(Tomado *et al* .,1996).** measurement of Antithrombin III was found to be a sensitive parameter for detection of latent clotting pathway activation.

Sudan is one of the developing countries, where pre-eclampsia is one of the main causes of obstetric complications with high maternal morbidities and mortality. Because of the importance of the disease for both mothers and babies along with the significant increase in the affected individuals among pregnant ladies, a reliable predictor for pre-eclampsia will play an important role in early prevention and intervention.

By doing so, the findings of this study could play an important role in decreasing morbidity and mortality of pregnant women induced hypertention . Furthermore, this study could be used as a reference or a benchmark study for related studies.

1.3 Objectives

1.3.1 General Objective

Associated of ATIII level ,PLT Count and PLT indices among Sudanese hypertensive pregnant women .

1.3.2 Specific Objectives

1. To estimate Antithrombin III level in pregnant women induced hypertention using Immunological assay by (ACCENT 200 Antithrombin III Kit) .
2. To evaluate Platelet Count and Indices in pregnant women induced hypertention using automated counter by (sysmex XP-300).
3. To compare between level of ATIII in normal pregnant women and pregnant women induced hypertention .
4. To compare between PLT Count and Indices in normal pregnant women and pregnant women induced hypertention .
5. To find out the possible association between age, contraceptive with the PLT Count , PLT indices and AT III .
6. To correlate between PLT count , PLT indices and AT III .

Chapter II

2.1 Literature Review

2.1.1 Pregnancy :

During pregnancy, the pregnant mother undergoes significant anatomical and physiological changes in order to nurture and accommodate the developing foetus. These changes begin after conception and affect every organ system in the body **(Locktich 1997).**

For most women experiencing an uncomplicated pregnancy, these changes resolve after pregnancy with minimal residual effects.

It is important to understand the normal physiological changes occurring in pregnancy as this will help differentiate from adaptations that are abnormal **(Priya *et al*.,2016).**

2.1.2 Haematological Changes Durig Pregnancy :

Plasma volume increases progressively throughout normal pregnancy **(Rodger 2015)** .Most of this 50% increase occurs by 34 weeks' gestation and is proportional to the birthweight of the baby. Because the expansion in plasma volume is greater than the increase in red blood cell mass, there is a fall in haemoglobin concentration, haematocrit and red blood cell count. Despite this haemodilution, there is usually no change in mean corpuscular volume (MCV) or mean corpuscular haemoglobin concentration (MCHC).

The platelet count tends to fall progressively during normal pregnancy, although it usually remains within normal limits.
In a proportion of women (5–10%), the count will reach levels of 100–150 ×109 cells/l by term and this occurs in the absence of any pathological process. In practice, therefore, a woman is not considered to be thrombocytopenic in pregnancy until the platelet count is less than 100 ×109 cells/l.
Pregnancy causes a two- to three-fold increase in the requirement for iron, not only for haemoglobin synthesis but also for for the foetus and the production of certain enzymes.

There is a 10- to 20-fold increase in folate requirements and a two-fold increase in the requirement for vitamin B12. Changes in the coagulation system during pregnancy produce a physiological hypercoagulable state (in preparation for haemostasis following delivery) **(Ramsay M 2010).**

The concentrations of certain clotting factors, particularly VIII, IX and X, are increased. Fibrinogen levels rise significantly by up to 50% and fibrinolytic activity is decreased. Concentrations of endogenous anticoagulants such as antithrombin and protein S decrease.

Thus pregnancy alters the balance within the coagulation system in favour of clotting, predisposing the pregnant and postpartum woman to venous thrombosis. This increased risk is present from the first trimester and for at least 12 weeks following delivery.

In Vitro tests of coagulation [activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT)] remain normal in the absence of anticoagulants or a coagulopathy.

Venous stasis in the lower limbs is associated with venodilation and decreased flow, which is more marked on the left. This is due to compression of the left iliac vein by the left iliac artery and the ovarian artery. On the right, the iliac artery does not cross the vein.

2.1.3 Pregnancy Induced Hypertention

Pregnancy Induced hypertension (PIH) is one of the most common and potentially life threatening complication of pregnancy. It affects 5-8 percent of all pregnancies and is one of the leading causes of high morbidity for both mother and foetus, especially in developing countries **(Vanderjagt *et al* .,2004)** . PIH is the development of hypertension in the second half of pregnancy on two or more occasions, about four hours apart, in a woman who previously been normotensive, and in whom blood pressure (BP) return to normal with six weeks of delivery. Pregnancy induced hypertension is essentially a disease of primigravidia and is more common in the age group of <20 and >35 pears. When the condition is present in multipara, it is commonly associated with multiple pregnancy, essential chronic hypertension and chronic renal disease. In a previous report

in Lagos on low birth weight babies, it was found that PIH complicated twin pregnancies in 21.8% of cases, almost twice the figure of 12.4% for singleton pregnancies **(Akingbol *et al* ., 2006)** . Pregnancy induced hypertension can be classified into; gestational hypertension or pregnancy induced hypertension alone without proteinuria. Pregnancy induced hypertension without intervention can progress to eclampsia, which is characterized by hypertension, proteinuria, oedema and epileptiform convulsions requiring emergency caesarean section **(Packer 2005)** . The importance of the problem is linked to the significant morbidity and mortality potential of pregnancy induced hypertension. The mother may develop disseminated intravascular coagulation, acute renal failure, stroke (ischaemia, due to vasospasm and microthrombosis or even haemorrhage due to severe thrombocytopenia), acute pulmonary oedema, cerebral oedema, placental abruption, liver haemorrhage/rupture, transformation in chronic hypertension, or even maternal death (preeclampsia is the second cause of maternal death linked to pregnancy) **(Lim *et al*., 2009)** ; the foetal sufferance seems to be due exclusively to the placental insufficiency and may include: pregnancy loss, foetal death inutero, intrauterine growth restriction, premature labour
(Mutter *et al*., 2009).
On a long term, a woman with a history of preeclampsia has a chance to repeat it at a future pregnancy and a higher cardiovascular risk **(Lykke *et al* ., 2009).** Also, a 2.5 times higher rate of dying by ischaemic cardiovascular disease **(VanWijk *et al* ., 2000)**
Pregnancy induced hypertension (blood pressure greater than 140/90) occurs before or after 20 weeks gestation with no proteinuria. The clinical manifestations of maternal preeclampsia are hypertension and proteinuria with or without coexisting systemic abnormalities involving the kidneys, liver, or blood. HELLP syndrome is a severe form of preeclampsia and involves haemolytic anemia, elevated liver function tests (LFTs), and low platelet count **(Steegers *et al* .,2010).**There is paucity of data on the effect of Pregnancy Induced Hypertension on the platelet count.

The aim of this case-control study was to investigate the effect of Pregnancy Induced Hypertension on the platelet count.

2.1.4 Preeclampsia

Preeclampsia is a clinical manifestation characterized by hypertension, proteinuria and edema that occurs after 20th week of pregnancy **(F. Gary *et al* ., 2010).**1 It is an idiopathic multisystem disorder of pregnancy affecting about 5-8% of all pregnancies and is a major cause of maternal, fetal and neonatal mortality and morbidity **(Stekkinger E *et al* ., 2009)** Although the etiology of preeclampsia remains unknown, it is suggested that preeclampsia is associated with intervillous and spiral artery thrombosis, vascular endothelial damage and abnormalities of coagulation, leading to inadequate maternal, fetal and placental circulation **(Roberts *et al* ., 2001).** Immunological adaptation disorders, abnormal increase of vasoconstrictor tone, nutritional factors, and genetic factors are some other theories **(Lopez-Jaramillo *et al* ., 2001)**.

Normal pregnancy is associated with changes in the haemostatic mechanism with increased levels of the coagulation factors and suppressing of fibrinolysis .

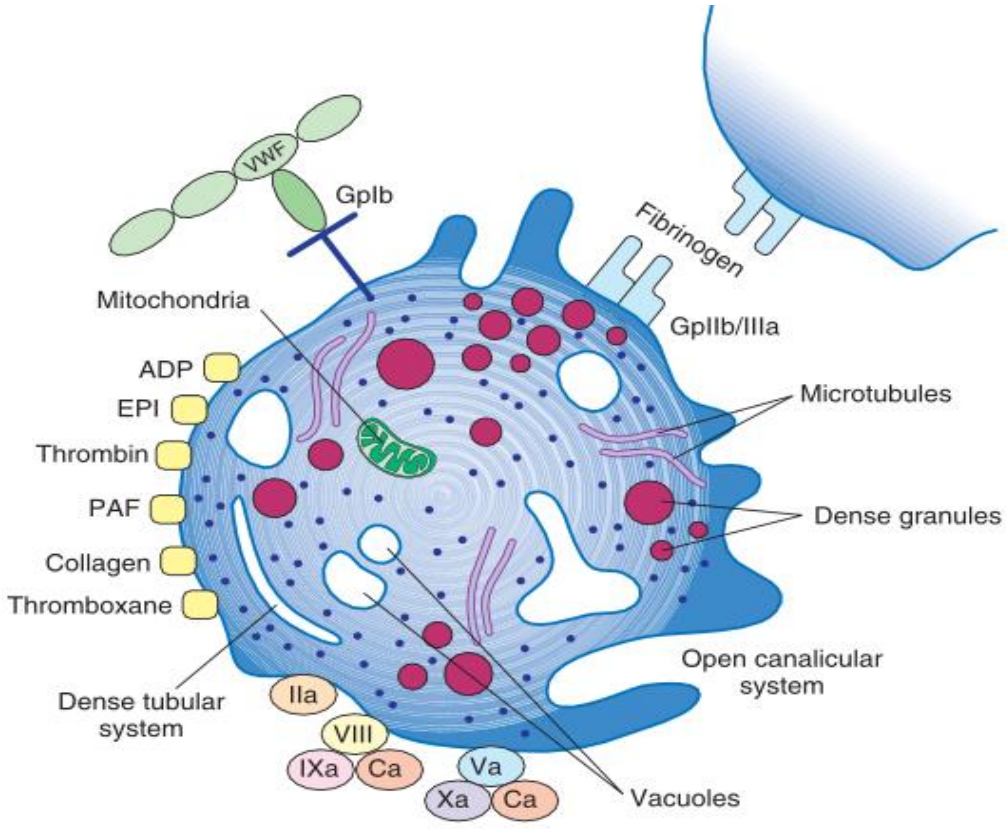
Therefore normal pregnancy is considered to be the state of hypercoagulability and hypercoagulation is more pronounced during the third trimester. Pregnancy is a state of silently ongoing intravascular coagulation at least in the uteroplacental circulation. These modification of the coagulation system is to ensure rapid and effective control of bleeding from the placental site and prevent fatal hemorrhage during delivery and puerperium **(Bremme 2003).**

Preeclampsia is a pathophysiological disorder specific to pregnancy and characterized by vasoconstriction and hypercoagulability. In women with preeclampsia, intravascular coagulation frequently coexists leading to thrombus formation in the uteroplacental unit and other maternal organs and thrombocytopenia or hemoconcentration are frequently associated with low AT-III activity in pathological conditions. The balance between coagulation and anticoagulation systems is shifted towards coagulation, and thus there is a thrombotic tendency. In preeclamptic pregnancies, the coagulation is generally activated with more decreased fibrinolysis, with platelet activation and consumption, promoting of thrombin formation and fibrin formation and destruction **(Helimann *et al* ., 2007) .**There is a shift in the haemostatic balance towards a pro-thrombotic state, together

with changes in endothelial and placental function **(Sibai *et al* ., 2005)** Recently measurements of various prothrombotic
markers have become available and the measurement of Antithrombin III was found to be a sensitive parameter for detection of latent clotting pathway activation.

2.1.5 Platelet :

Platelets are small a nucleate cell fragments that have a characteristic discoid shape and range from 1 to 3 nm in diameter . PLT are indispensable for processes such as hemostasis , wound healing , angiogenesis , inflammation , and innate immunity. Platelets are formed from the cytoplasm of megakaryocytes (MKs), their precursor cells ,which reside in the bone marrow . MKs are the largest (50-100 nm) and also one of the rarest cells in the bone marrow . **(Machlus 2013)**



activation (Hoffbrand and Moss,2016).

Figure 2.1: Schematic diagram of platelet morphology (Ciesla 2007).

2.1.5.1 Platelet Counting

Platelets can be counted in whole blood using the techniques of impedance counting (electrical) or light scattering (electro-optical) detection. An upper threshold is needed to separate platelets from red cells and a lower threshold is needed to separate platelets from debris and electronic noise. Recirculation of red cells near the aperture should be prevented, because the pulses produced may simulate those generated by platelets. Three techniques for setting thresholds have been used: (1)platelets can be counted between two fixed thresholds (e.g.between 2 and 20 FL); (2)pulses between fixed thresholds can be counted with subsequent fitting of a curve and extrapolation so that platelets falling outside the fixed thresholds are included in the computed count; and (3)thresholds can vary automatically, depending on the characteristics of individual blood samples, to make allowance for microcytic or fragmented red cells or for giant platelets. Factitiously low impedance platelet counts may be the result of giant platelets being identified as red cells or of EDTA-induced platelet clumping or satellitism. Misleadingly, high platelet counts may be due to markedly microcytic or fragmented red cells, to white cell fragments in leukaemia or to bacteria or fungi **(Bain BJ *et al* .,2016).**

2.1.5.2 Platelet Indices

Complete blood count (CBC) tests with automated haematology analysers are one of the most commonly ordered tests in clinical laboratories. Modern haematology analysers in routine diagnostic use, which measure platelet indices (PIs), use impedance counting or optical light scatter counting techniques. The measurement principle influences the results, and the results from different analysers are not comparable **(Lippi *et al* .,2016)** .

Platelet count in the blood can be rapidly measured using an automated haematologic analyser.

Platelet indices are biomarkers of platelet 12 activation. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings without bringing extra costs. Among these platelet indices, plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) are a group of platelet

parameters determined together in automatic CBC profiles; they are related to platelets' morphology and proliferation kinetics .

The volume of platelets in the bloodstream is heterogeneous, and their structures and metabolic functions differ. Typically, the average mean cell volume is 7.2–11.7 fL in healthy subjects **(Demirin *et al* ., 2011) .**

In MPV, the analyser calculated measure of thrombocyte volume is determined directly by analyzing the platelet distribution curve, which is calculated from a log transformation of the platelet volume distribution curve, to yield a geometric mean for this parameter in impedance technology systems. In some optical systems, MPV is the mode of the measured platelet volume **(Senaran *et al* .,2001).**

MPV is determined in the progenitor cell, the bone marrow megakaryocyte. The platelet volume is found to be associated with cytokines (thrombopoietin, interleukin-6 and interleukin-3) that regulate megakaryocyte ploidy and platelet number and result in the production of larger platelets **(Larsen *et al* ., 2014) .**

When platelet production is decreased, young platelets become bigger and more 13 active, and MPV levels increase. Increased MPV indicates increased platelet diameter, which can be used as a marker of production rate and platelet activation. During activation, platelets' shapes change from biconcave discs to spherical, and a pronounced pseudopod formation occurs that leads to MPV increase during platelet activation. PDW is an indicator of volume variability in platelets size and is increased in the presence of platelet anisocytosis **(Osselaer *et al* .,1997).**

PDW is a distribution curve of platelets measured at the level of 20% relative height in a plateletsize distribution curve, with a total curve height of 100% (Sachdev R, et al,2014). The PDW reported varies markedly, with reference intervals ranging from 8.3 to 56.6% **(Maluf *et al* ., 2015).**

PDW directly measures variability in platelet size, changes with platelet activation, and reflects the heterogeneity in platelet morphology **(Vagdatli *et al* ., 2010).**

Under physiological conditions, there is a direct relationship between MPV and PDW; both usually change in the same direction **(Vagdatli *et al* .,2010).**

Meanwhile, there are conflicting reports in the literature about the relationship between platelet volume and numbers, which suggests that they are affected by different

mechanisms .PCT is the volume occupied by platelets in the blood as a percentage and calculated according to the formula PCT = platelet count × MPV / 10,000 **(Chandrashekar 2013).**

Under physiological conditions, the amount of platelets in the blood is maintained in an equilibrium state by regeneration and elimination. The normal range for PCT is 0.22–0.24% **(Wiwanitkit 2004).**

In healthy subjects, platelet mass is closely regulated to keep itconstant, while MPV is inversely related to platelet counts **(Margetic 2012).**

Genetic and acquired factors, such as race, age, smoking status, alcohol consumption, and physical activity, modify blood platelet count and MPV **(Hong *et al* ., 2015).**

2.1.6 Antithrombin III

AT III is a glycoprotein synthesized in the liver, with a molecular weight of 58,000 and a plasma half-life of about 67 hour. It inhibits action of thrombin and other activated coagulation factors namely Xa, IXa, XIa, XIIa, plasmin and kallikrein. Its action is potentiated by heparin. It is the most critical modulator of coagulation and has potent anti-inflammatory properties independent of its effects on coagulation **(Nwogoh Benedict *et al* .,2016).**

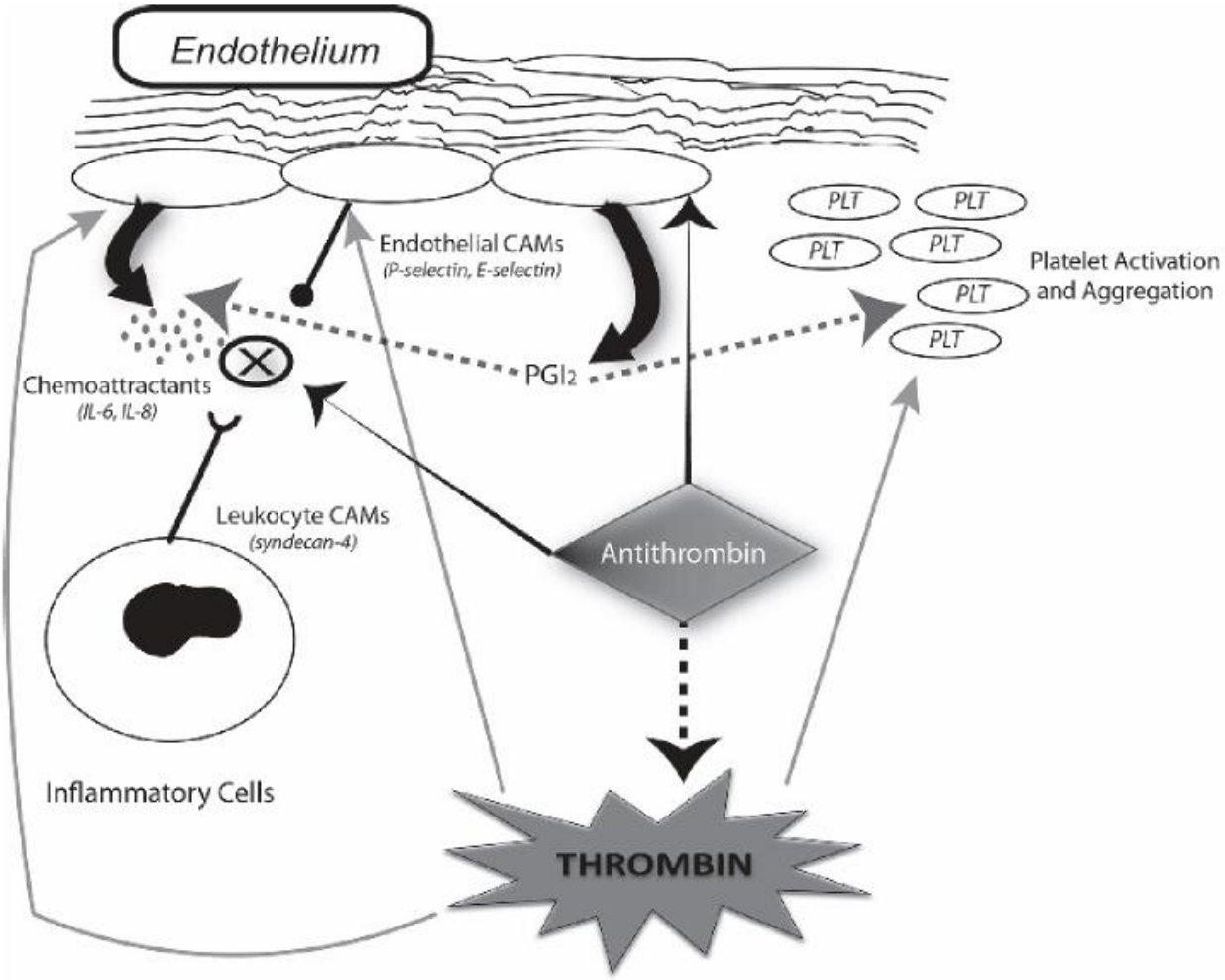


Figure 2.2: antithrombin action (Jerrold *et al.*, 2016).

2.1.6.1 Pathophysiology of Antithrombin

Role of Antithrombin111 in coagulation Antithrombin111 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. Antithrombin111 physiologically circulates in a form that has a low inhibitory activity. The anticoagulant effect of Antithrombin111 is accelerated at least a thousand times in the presence of heparin and other heparin-like glycosaminoglycans, such as heparin sulphate. As free heparin is not present in the circulation under normal physiological circumstances, it is thought that the heparin sulphate located on the vascular endothelium provides the main backbone for this accelerating mechanism. The therapeutic use of heparin as an anticoagulant works through the potentiation of endogenous Antithrombin111. The Antithrombin111 - mediated inactivation process for coagulation factors requires the binding of a unique sequence- specific pentasaccharide domain of heparin to the heparin-binding domain of Antithrombin111. This interaction induces a conformational change in Antithrombin111, which accelerates the inhibition of FXa. The inhibition of thrombin, in addition, requires heparin to bind to both Antithrombin111 and thrombin, to form a ternary bridging complex, so that then thrombin can be inhibited. The proposed sequence of events is that Antithrombin111 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 Antithrombin111 undergoes a slow conversion to a latent form that is not only inactive by itself but also dimerizes with an active Antithrombin111 molecule (with preferential binding to the b-isoforms of Antithrombin111). 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 Antithrombin111 (Rouen-VI AT variant) during febrile episodes. In addition to its anticoagulant role, AT 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, Antithrombin111 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 Antithrombin111 are closely dependent on its ability to bind with the endothelial glycosaminoglycans and are not observed in its reaction with commercial/free circulating heparin .

2.1.6.2 Classification of Antithrombin III deficiency

Heritable Antithrombin III deficiency

Two major phenotypes of heritable Antithrombin111deficiency are recognized. Type I is characterized by a quantitative reduction of qualitatively normal Antithrombin111. Type II deficiency is due to the production of a qualitatively abnormal Antithrombin111 protein. In both types of Antithrombin111 deficiency, Antithrombin111 activity is reduced to a variable extent. In type I deficiency Antithrombin111 antigen levels are reduced concordantly with the functional reduction. In type II deficiency, Antithrombin111 antigen levels are discordantly higher than the functional levels and may be close to normal. Understanding of the basis of familial Antithrombin111 deficiency has been facilitated by advances made in molecular biology and in the functional characterization of this inhibitory glycoprotein. The Antithrombin111 molecule possesses two important functional regions – a heparin-binding domain and a thrombin-binding domain. Type II Antithrombin111 deficiency is sub-classified according to the site of the molecular defect, Lane classified this type of deficiency in more variants ; **type II RS** (realixe site or reactive site) included mutations which affect the aminoacids of the clivation zone of the AT III by thrombin (between Arg 393 and Ser 394) or some adjacent aminoacids. **Type II HBS** (heparin binding site) is independent of the interaction AT III-heparin.**Type II PE** (pleiotropic effect) - multiple mutations result in abnormalities to the reactive site as well as binding sites. Many mutations associated with Antithrombin111 deficiency have been described, but identification of the specific mutation in particular patients is neither practical nor necessary for clinical purposes.

Phenotypic distinction between the subtypes of Antithrombin111 deficiency is, however, of clinical relevance as the incidence of thrombosis is higher in association with type I deficiency and type II deficiency when the mutation affects the reactive site than in type II deficiency when the mutation affects the heparin binding site. Type II HBS variants, although associated with a lower risk of thrombosis than type II RS defects, may increase the attributable risk of an additional thrombophilic defect, such as the FV Leiden mutation. Heritable Antithrombin111 deficiency is uncommon, and type II deficiency is more prevalent than type I deficiency. The prevalence's of heterozygous type I and type II mutations are approximately 0.02% and 0.15% respectively. **(Oven E *et al* .,2006).**

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 Antithrombin111deficiency are exposed to a high risk of thromboembolism, due to depletion of a protein critical to anticoagulation in plasma. Low Antithrombin111levels could be detected not only during but also before the thrombotic event. Acquired Antithrombin111 deficiency occurs in different medical conditions with a similar risk of thrombosis **(Zeyuan *et al* ., 2017).**

2.1.6.3 Clinical signs and symptoms

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).**

2.1.6.4 Laboratory Evaluation of Antithrombin III

Antigenic Assays The first assays developed for detection of Antithrombin111 deficiency quantified the antigenic form of the molecule by radio immunodiffusion techniques or Laurell rocket electrophoresis.The immunologic methods are not widely used at the present time, but are useful for detection of patients with the rare type II defect. 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 Antithrombin111. While these methods are very specific, their sensitivity is limited. However, since most patients with congenital defects have about a 50% decrease in protein level it is usually possible to obtain a correct result. Coefficients of variation (CVs) for radial immunodiffusion or other immunological methods are much larger than chromogenic substrate as says. **Functional Assays** Almost all current methods measure functional levels of the Antithrombin111protein by use of synthetic substrate technology using predominantly amidolytic methods. This technique employs a synthetic peptide that mimics the natural target substrate of the enzyme, which is attached to a chromogenic group at or near the cleavage site. In this assay, patient plasma is incubated with an excess of thrombin in the presence of heparin. In the first phase of the reaction, the Antithrombin111neutralizes the thrombin in the presence of heparin. The remaining thrombin, which is inversely proportional to the amount of Antithrombin111in the patient plasma, is then quantitied by the cleavage of para-nitroaniline from the peptide substrate at 405 nm. The assay is easily automated and can be performed on either a coagulation analyzer or almost any chemistry instrument. Some assays use inhibition of factor Xa rather than thrombin to decrease the contribution from other proteins, such as heparin cofactor II. Since most amidolytic assays have the potential for nonspecificity due to substrate cleavage by other proteases, the newer commercial Antithrombin111assays have protease inhibitors, such as aprotinin, which minimizes nonspecific substrate cleavage, and bovine thrombin, which is resistant to any impact of heparin cofactor II. The amidolytic Antithrombin111 assays are very sensitive and specific, and are not influenced by the presence of heparin in the patient plasma. Most amidolytic assays are not able to distinguish patients with type IIb deficiency from the other categories, but this distinction is of doubtful clinical

significance, except that individuals with type IIb deficiency may have a lower risk of thrombosis. Inter assay CV percentages of 3% to 5% are possible, although CV percentages of approximately 10% were observed in a recent College of American Pathologists survey. The assay has a positive predictive value of 96% in patients with congenital Antithrombin111deficiency states. A 2-SD normal range for Antithrombin111activity obtained from 9669 normal blood donors was determined to be 83% to 128%. The normal range was not significantly different for males or females, in females on oral contraceptive therapy, during pregnancy, or with smoking. Antithrombin111levels are lower in neonates and increase to adult ranges by approximately 1 year; levels then are slightly increased compared to adult levels up until approximately age 16 years. Recent assay developments include the description of a global clotting-based assay for the factor II/ Antithrombin111 system and the development of a clot-based assay for Antithrombin111 quantitation, which uses A heparinzed Antithrombin111- deficient plasma and is performed exactly like a traditional coagulation factor assay. These types of assays may be useful in laboratories that do not have instrumentation to perform chromogenic assays. Prior clotting Antithrombin111 assays were difficult to perform and subject to variability as they used thrombin, so the plasma had to be defibrinated before testing or a serum sample was used.

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. Proper performance of functional Antithrombin111 assays should also include consideration of preanalytic variables, establishment of an accurate reference range, and validation of the calibrator used to establish the standard curve. Due to ex vivo coagulation activation, coagulation testing specimens may be plagued by preanalytic variables, so careful collection and processing is required. Samples that are hemolyzed, lipemic, clotted, from individuals with a hematocrit greater than 55%,or that are underfilled are not acceptable for performing Antithrombin111 assays or coagulation testing in general. Laboratory-specific reference ranges should be established by analysis of plasma samples from at least 40 normal individuals by calculating the population means 62 SD from the mean. Calculation of laboratory-specific reference ranges is important, owing to variability in

test methodology from site to site and variability in population parameters. Most amidolytic Antithrombin111 results are calculated from a standard curve constructed using serial dilutions of a normal plasma calibrator, with results reported as percentage of normal. Manufacturers usually calibrate these plasmas against the current Antithrombin111 Standard from the National Institute for Biological Standards and Control, but laboratories should validate that the Antithrombin111 activity of the calibrator provided by the manufacturer is accurate. **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. Controversy still surrounds older thrombophilia patients (50 years) with no family history or individuals with thrombosis associated with a predisposing event. Given that almost all Antithrombin111 -deficient patients will have a thrombosis before this age, as well as the relative rarity of the condition, some clinicians would argue that it is not justified either on scientific or cost grounds to test such patients for Antithrombin111 deficiency or any other congenital thrombophilic disorders. Much of this controversy relates to the fact that the thrombosis itself will be treated in the same way, regardless of whether the patient has an identified etiology. Almost all data indicate that testing patients for thrombophilia markers in the acute phase of a thrombotic event is not appropriate, given the variable consumption of factors like Antithrombin111 and protein C during such times. However, in modern hospital practice with immense pressure for short or no hospitalization for venous thrombosis patients, for example, it may be the only time the patient can be tested before several months of anticoagulant therapy. If Antithrombin111 testing is carried out during this time and yields normal results, it is unlikely the patient is Antithrombin111 deficient. However, if the result is only modestly abnormal, then no diagnostic conclusion can be drawn, since intravenous un-fractionated heparin therapy itself can cause lowering of the Antithrombin111 level in plasma by about 25%. In an ideal situation, the patient should be evaluated at least 3 months after their event to determine potential Antithrombin111 deficiency. The

algorithm in may be helpful in establishing a diagnosis of Antithrombin111 deficiency. If the initial Antithrombin111 level is normal or elevated, Antithrombin111 deficiency is unlikely. However, Antithrombin111 levels may be increased in some patients on oral anticoagulation therapy, which could potentially mask an Antithrombin111 deficiency. If the initial Antithrombin111 level is low, then a confirmatory Antithrombin111 test should be done on the patient using a repeat specimen. It may also be helpful to test first-degree relatives for Antithrombin111 deficiency. These studies should confirm the Antithrombin111 deficiency before the patient is considered to be truly Antithrombin111 deficient. No patient should be diagnosed on 1 assay alone, no matter how profound the Antithrombin111 deficiency. Acquired causes of Antithrombin111 deficiency should be excluded. On the repeat specimen, both activity and immunological assays may be carried out to determine if the patient has a type I or type II Antithrombin111 deficiency. However, since the anticoagulant therapy is the same for both type I and type II Antithrombin111 deficiencies, the sub-classification is not clinically necessary, except to address familial or epidemiologic concerns. Further characterization of the actual gene mutation is rarely performed because of lack of clinical necessity and the large number of potential mutations. Genetic identification of type 11b may be of epidemiologic interest, as it is associated with a lower thrombosis risk because the mutations do not involve the active site, but rather involve the heparin- binding site. In patients with acquired Antithrombin111deficiency, the use and frequency of Antithrombin111testing will depend on the clinical situation. Patients with active disseminated intravascular coagulation may require testing every hour if they are actively bleeding, whereas a patient with a more indolent disease state, such as nephrotic syndrome, may require testing much more infrequently **(Kandice K., et al 2002).** Many techniques for the analyses of AT-III genetic alterations have been developed previously. The most sensi-tive mutation detection technique is considered to be direct sequencing; however, the sequencing of AT-III gene is technically demanding, time consuming, and costly. Thus, multiple techniques have been employed to screen DNA sequence alterations in order to reduce the amount of DNA sequencing needed. These techniques include SSCP, DGGE, etc. Each method has its own limitations, which range from labor intensive and time consuming to high reagent costs and sensitivity or specificity issues. Denaturing high-

performance liquid chromatography is a highly sensitive and specific method for detecting an unknown sequence variation. With the optimal running conditions, all variants in a PCR segment could be detected **(Li-Ping *et al* ., 2010).**

2.1.6.5 Some cases patients should not be investigated for Antithrombin111deficiency

• In patients who are receiving a vitamin K antagonist, ATIII levels will be Substantially decreased and this is an acquired (and expected) finding.

• In patients who are pregnant or taking an oral contraceptive, ATIII levels will be mildly to moderately decreased **(Lipe *et al* ., 2011).**

2.1.6.6 Treatment of Antithrombin III

Treatment of Antithrombin111 deficiency is made with:

Antithrombin111 concentrates contain 1000 UI of Antithrombin111 (KiberninR HS 1000) Administration of 50 UI of Antithrombin111 /Kg increases plasmatic concentration of Antithrombin111 and reach approximately a 120% value at a heterozygous patient with a initial plasmatic concentration of 50%. The plasmatic level of Antithrombin111 must be maintained at levels above 80%. Treatment with concentrates of Antithrombin111 is specific for the patients which faced surgical interventions and pregnant women with Antithrombin111 deficiency (AT III concentrate + heparin prophylaxis). **Heparin therapy** is for all patients with Antithrombin111 deficiency, including pregnant women with risk of embriopathy at these preparates. Women with Antithrombin111 deficiency should avoid administration of oral contraceptives. In the context of frequent thrombotic accidents at a young age and decreased level of Antithrombin111 the established diagnosis was of deficiency of Antithrombin111; familial study showed an inherited nature of deficiency (patient's father was heterozygous but without thrombotic accidents and the descendants of the patient, aged between 2-6 years, presenting a 50% of deficiency of Antithrombin111, without thrombosis until those ages. The prominence of the deficiency of Antithrombin111 heterozygous form at the patient as well as his descendants is important for the prevention of thrombotic accidents in the future **(Amelia *et al* ., 2014).**

2.1.6.7 Management of Antithrombin111 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).**

2.2 previous studies

Study performed by **Onuigwe *et al* ., 2015** in blood sample from seventy (70)pregnant women with hypertension and thirty (30) subjects without complication in pregnancy serve as control ,blood collected and the number of platelets per litre of blood was calculated .Study observed that pregnancy induced hypertension (PIH) is significantly associated with low platelet count and that teenage pregnancy is significantly associated with low platelet count among patients with PIH.

Study by **Zamir Damani in 2016** , twenty (20) pregnant women with hypertension (HTA) and twenty (20) subjects without HTA as a control visited Hospital University of "Koço Gliozheni" Tirana, Albania ,EDTA blood sample were collected , platelets were counted using improved Neubauer ruled counting chamber (Hawksley, UK). Significant lower platelet count was observed among pregnant women with PIH compared to individuals from control group.

Study performed by **Eman *et al* ., 2013**. 150 pregnant women who were attending to Al -Zahraa University Hospital were divided into three groups: Normal pregnant women (n = 50) as a control group, pregnant women with mild PE (n = 50) and pregnant women with severe PE (n = 50). Preeclampsia was defined as elevated blood pressure of ≥ 140 / 90 mmHg after 20 weeks gestation with proteinuria ≥ 300 mg / 24 hours urineor > 1+ dipstick.Blood samples were collected upon admission in tubes with potassium ethylene diamine tetra acetate (EDTA) as an anticoagulant. The platelet count and platelet indices were estimated using the Sysmex Xe-2100 automated quantitative hematology analyzer . Result ; platelet count was significantly lower in women with severe PE compared to women with mild PE and normal pregnant women groups , Mean platelet volume and platelet width distribution were significantly higher in women with severe PE compared to women with mild PE and normal pregnant women groups.

Study performed by **Nooh *et al* ., 2015 .** observational longitudinal study of women attending antenatal clinic (ANC) and/or admitted to maternity ward at

Zagazig University Hospital (ZUH), Zagazig, Egypt over the period from 2nd June 2014 to 28th May 2015 , Data analyzed , observe that Platelet count (PC) was decreasing while mean platelet volume (MPV) and platelet distribution width (PDW) were increasing as PE progressed.

Study performed by **RAMALAKSHMI *et al* ., 1995** fasting blood samples were obtained from 45 pregnant women with PIH (systolic Blood pressure > 130 mmHg and/or diastolic blood pressure >90 mmHg, oedema and proteinuria) and 18 women with normal pregnancies matched for gestational age.Plasma Was separated and AT III Levels were estimated ,observed that Mean Antithrombin III levels Were lower in pregnancy induced hypertension compared to a control group of women.

Study by **Sarkar *et al* ., 2013** was undertaken to determine the changes in the levels of plasma Antithrombin -III(AT III) and platelet count in preeclamptic women and its comparison with healthy non-pregnant women and normal pregnant women 40women with preeclampsia in the third trimester of pregnancy constituted the study group. The study group was further divided into two subgroups as mild and severe preeclampsia. Age and gestational age matched 23 healthy non pregnant and 28 normal pregnant women were taken as a control group. systolic and diastolic blood pressure was observed. When compared with control groups (healthy non pregnant and normal pregnant women), the levels of AT III in preeclamptic group was significantly lower. Although there was no significant difference in the levels of AT III between healthy non pregnant and normal pregnant .

Study by **Mohamed ,F.A.N (2016)** in Sudan found a highly significant lower of PLT count in Pregnant with Preeclampsia compared to normal pregnant , Mean platelet volume and platelet width distribution were significantly higher in women with Preeclampsia compared to normal pregnant.

This study suggested that, platelet count, mean platelet volume (MPV), platelet distribution width (PDW) and other parameter can serve as early monitoring markers for the severity of pre-eclampsia .

Chapter III

3. Materials and Methods

3.1 Study design

This study was an analytic prospective Case-Control study was conducted in a period between January -Octoper 2019.

3.2 Study area

The study performed in Khartoum state in Dr.Magdy Lewise Clinic ,Albraha Hospital , Shefaee Clinic Center and Noor Al Hoda Modern Specialist Medical Center .

3.3 Study population

Eighty blood samples collected from volunteer women with normal pregnancy and induced hypertention pregnant women .

3.4 Inclusion Criteria

- 40 Sudanese Healthy pregnant women .
- 40 Sudanese pregnant women induced hypertention .

3.5 Exclusion criteria

- Mother presents with coagulation diseases under treatment such as anticoagulant therapy , other medical conditions that can affect the result were excluded from this study such as blood or Platelet transfusion ,liver disease , Mixed Infection etc.

3.6 Ethical considerations

Ethical committee of research in the Sudan University of Science and Technology, Faculty of Medical Laboratory Science 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.

3.7 Data Collection

Data collected using informed consent and designed written questionnaire to obtain information about demographical and clinical data that helped in either including or excluding certain subjects.

3.8 Sampling and Sample size

Sampling method was a simple random sampling technique. A total of 80 samples (40 cases and 40 controls) were collected.

3.8.1 Sample collection

2.7 ml of venous blood was collected from individual under study and dispensed in Tri Sodium Citrate (1ml of TSC : 9 ml of blood) container blood mixed gently for avoidance of clotting and hemolysis separated by using centrifuge 2500 rpm for 15-minute lead to formation of platelet poor plasma (PPP) , for measurement of Antithrombin III by using ACCENT 200 Antithrombin111 Kit .

3 ml of venous blood sample was collected in ethylenediaminetetra-acetate (EDTA) evacuated tube, blood mixed gently for avoidance of clotting and hemolysis , to measure PLT Count and Indices by using automated hematological analyzer (sysmex XP-300).

3.8.2 sample technique

The control cases were randomly selected from pregnant women who attended the hospitals and Clinics for routine obstetric care using simple random sampling from the list of all pregnant women who attended to the hospitals during the same time.

The hypertensive cases that were found in the hospitals and clinics during data collection period were included .

3.8.3 Hematological techmique

3.8.3 Sysmex X 300

Automated hematological analyzer measures the cell counts using direct current (DC) detection method

3.8.3.1 Principle

Blood sample is aspirated, measured to predetermined volume, diluted at the specified ratio, and then fed into each transducer.The TD chamber has a minute hole called the aperture. On both sides of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes.

As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses.

3.8.3.2 Procedure of Plts Count and Plts indecis

As PLT lower discriminator (LD) and upper discriminator (UD) and the fixed discriminator used for discriminating platelet sizes , the optimum position in 2 – 6 fl and 12 – 30 fl, respectively, were automatically determined by the microcomputer. PLT were calculated from the particle counted between this three discriminators .

PDW is the distribution width on 20% frequency level with the peak taken as 100%. The unit applied was femtoliter (fl).

MPV was calculated by the following formula: MPV (fl) = PCT (%) × 1000 / PLT (103/μl)

Where PCT (%) represented the value weight with platelet frequency and is called platelet- crit or platelet volume ratio.

3.8.3.2.1 Q.C of Sysmex

- The reliability of the **Sysmex** and reagents is monitored by quality control. By use of control or blood or control materials the stability of the measured value is monitored over a certain period of time, and problems can be detected early or prevented.

3.8.4 AT III Kit

3.8.4.1 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 340nm is proportional to Antithrombin III concentration in the sample.

3.8.4.2 Component

Package

1-Reagent 1 x 35 ml

2-Reagent 1 x 7 ml

Buffer (1-Reagent) stored at 2-25□C and antiserum (2-Reagent) stored at 2-8□C are stable until expiry date printed on the package. Store closed and avoid contamination.

3.8.4.3 Q.C

- For internal quality control it is recommended to use the CORMAY IMMUNO-CONTROL III (Cat. No 4-291) with each batch of samples. For the calibration of automatic analyzers systems the CORMAY 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).
- Avoid excessive mixing of the blood with the anticoagulant since the Hb affect in the result of AT III.

3.8.5 Data analysis

The data obtained from the automated mindray BS-200 machine and **Sysmex XP-300** automated hematological analyzer was entered and analyzed by using Statistical Package for Social Science (SPSS) version 23 (SPSS INC, Chicago, IL, USA).

3.8.5.1 Statistical analysis

The mean and standard deviation were used to summarize Antithrombin III level , PLT Count and indices . Independent T test (analysis of variance) was done to compare the mean AT , PLT and indices difference across the two groups hypertention and Normotension in difference Age with other variables.*P-value* of <0.05 was considered as statistically significant. Tables and figures were used for the description of the data.

Chapter IV

4. Results

4.1 Demographic Data

A total of 80 blood samples from Sudanese volunteers pregnant women 40 of them induced hypertention and matched with 40 healthy pregnant women as control enrolled in this study, subdivided into two group ranges , 42.5% of pregnant women aged 18-30 years and 57.5 % of them where 31- 42 years .

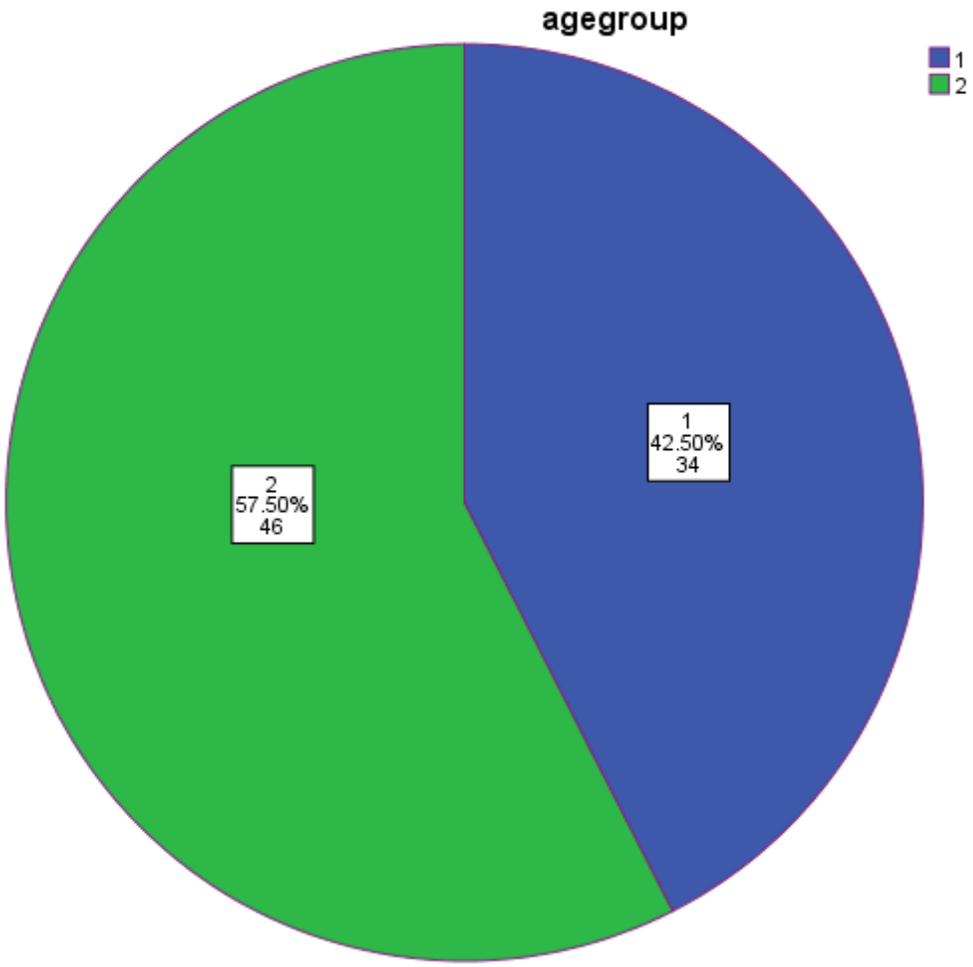


Figure (4.1) distribution of patients according to age group

Also 18 % of them were using contraceptives whereas 3 % of them were not using .

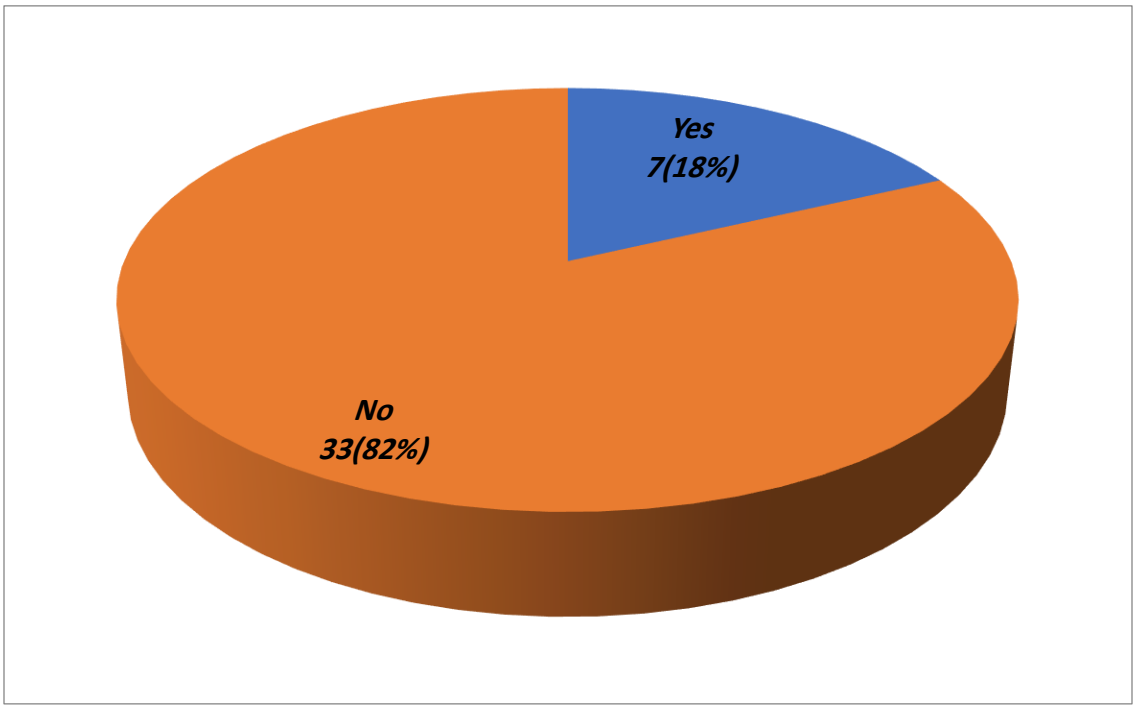


Figure (4.2) distribution of patients according to Contraceptive

4.2 mean comparison of AT III , PLT Count , MPV , PDW and PCT in case versus control group

Mean ± SD of ATIII level of induced hypertention pregnant women 0.22±0.03 mg / dl , While , mean ± SD of ATIII level of normal pregnant women 0.32±0.06 . In addition, there is a high significant decrease of AT III level in pregnant women induced hypertention in compare with AT III level in normal pregnant women (P. value = 0.00). Mean ± SD of PLT Count of induced hypertention pregnant women 188±41.9 (109L) , While , mean ± SD of PLT Count of normal pregnant women 233±39.1 (109L), there is a high significant decrease of PLT Count of induced hypertention pregnant women in compare with PLT Count of normal pregnant women (P. value = 0.00). Mean ± SD of MPV of induced hypertention pregnant women 11.37±1.53 (fl) , While , mean ± SD of MPV of normal pregnant women 10.45±1.58 (fl), there is a significant increased of MPV of induced hypertention pregnant women in compare with MPV of normal pregnant women (P. value = 0.01). Mean ± SD of PDW of induced hypertention pregnant women 16.25±1.86 (fl) , While , mean ± SD of PDW of normal pregnant women 15.29±2.04 (fl)), there is a significant increased of PDW of induced hypertention pregnant women in compare with PDW Count of normal pregnant women (P. value = 0.030). Mean ± SD of PCT of induced hypertention pregnant women 0.21±0.05 (%). While , mean ± SD of PCT of normal pregnant women 0.24±0.06, there is a significant decrease of PCT of induced hypertention pregnant women in compare with PCT of normal pregnant women (P. value = 0.015). (Table 3.1 represents the results).

Table (4.1) mean comparison of study parameters in case versus control group

Parameters	Case (Mean±SD)	Control (Mean±SD)	*P-value*
ATIII mg / dl	0.22±0.03	0.32±0.06	0.000
PLT (109L)	188±41.9	233±39.1	0.000
MPV (fl)	11.37±1.53	10.45±1.58	0.010
PDW (fl)	16.25±1.86	15.29±2.04	0.030
PCT (%)	0.21±0.05	0.24±0.06	0.015

4.3 mean comparison of AT III , PLT Count , MPV , PDW and PCT across age group

Mean ± SD of ATIII level of induced hypertention pregnant women with age 18-30 years 0.27±0.07 mg/dl, While , mean ± SD of ATIII level of age 31-42 years 0.27±0.07 (P. value = 0.84).

Mean ± SD of PLT Count of induced hypertention pregnant women with age 18-30 years 210.18±38.66 (109L) , While , mean ± SD of PLT Count of age 31-42 years 210.85±51.44 (109L) (P. value = 0.95).

Mean ± SD of MPV of induced hypertention pregnant women with age 18-30 years 10.7±1.63 (fl) , While , mean ± SD of MPV of age 31-42 years 11.06±1.60 (fl) (P. value = 0.31).

Mean ± SD of PDW of induced hypertention pregnant women with age 18-30 years 15.71±2 (fl) , While , mean ± SD of PDW of age 31-42 years 15.71±2 (fl) (P. value = 0.82).

Mean ± SD of PCT of induced hypertention pregnant women with age 18-30 years 0.22±0.05 (%) , While , mean ± SD of PCT of age 31-42 years 0.23±0.07 (%) (P. value = 0.45).

Table (4.2) mean comparison of study parameters across age group

Parameters	18 - 30 Years (Mean±SD)	31 - 42 Years (Mean±SD)	*P-value*
ATIII	0.27±0.07	0.27±0.07	0.84
PLT	210.18±38.66	210.85±51.44	0.95
MPV	10.7±1.63	11.06±1.60	0.31
PDW	15.71±2	15.81±2	0.82
PCT	0.22±0.05	0.23±0.07	0.45

4.4 mean comparison of study parameters across Contraceptive

Mean ± SD of ATIII level of induced hypertention pregnant women whom was using contraceptive 0.25±0.01 mg/dl, While , mean ± SD of ATIII level of those whom was not use 0.27±0.07 mg/dl. That mean there was no statistically significant difference in mean Antithrombin111 level when compared in women using contraceptives and not using contraceptives (P. value = 0.068).

Mean ± SD of PLT of induced hypertention pregnant women whom was using contraceptive 218.1±67.2 (109L), While , mean ± SD of PLT level of those whom was not use 209±44.3 (109L). there was no statistically significant difference PLT Count of induced hypertention pregnant women using contraceptives compared with those whom was not use (P. value = 0.652).

Mean ± SD of MPV of induced hypertention pregnant women whom was using contraceptive 11.29±1.13 (fl) , While , mean ± SD of MPV of those whom was not use 10.87±1.65 (fl) . there was no statistically significant difference in MPV when compared in women using contraceptives and not using contraceptives (P. value = 0.518).

Mean ± SD of PDW of induced hypertention pregnant women whom was using contraceptive 16.73±1.67 (fl) , While , mean ± SD of PDW of those whom was not use 15.68±2.02 (fl). there was no statistically significant difference in PDW when compared in women using contraceptives and not using contraceptives (P. value = 0.186).

Mean ± SD of PCT of induced hypertention pregnant women whom was using contraceptive 0.24±0.07 (%), While , mean ± SD of PCT of those whom was not use 0.23±0.06 (%) . there was no statistically significant difference in PCT when compared in women using contraceptives and not using contraceptives (P. value = 0.460). (Table 3.3 represents the results).

Table (4.3) mean comparison of study parameters across Contraceptive

Parameters	Yes (Mean±SD)	No (Mean±SD)	*P-value*
ATIII mg/dl	0.25±0.01	0.27±0.07	0.068
PLT (109L)	218.1±67.2	209±44.3	0.652
MPV (fl)	11.29±1.13	10.87±1.65	0.518
PDW (fl)	16.73±1.67	15.68±2.02	0.186
PCT (%)	0.24±0.07	0.23±0.06	0.460

4.5 Correlations between PLT , PLT indices and AT III

There is moderate positive correlation between AT III and PLT count , a week negative correlation between AT III and MPV , week negative correlation between AT III and PDW ,a week positive correlation Between AT III and PCT.
Week negative correlation between PLT and MPV has been found ,a week negative correlation between PLT and PDW ,a strong positive correlation between PLT and PCT.
Week positive correlation between MPV and PDW , week positive correlation between MPV and PCT.
Week positive correlation between PDW and PCT.

Table (4.4) Correlations between PLT , PLT indices and AT III

		ATIII	PLT	MPV	PDW	PCT
ATIII	Pearson Correlation	1	.438**	-.261*	-.344**	.213
	Sig. (2-tailed)		.000	.019	.002	.058
	N	80	80	80	80	80
PLT	Pearson Correlation	.438**	1	-.193	-.054	.759**
	Sig. (2-tailed)	.000		.087	.632	.000
	N	80	80	80	80	80
MPV	Pearson Correlation	-.261*	-.193	1	.094	.383**
	Sig. (2-tailed)	.019	.087		.406	.000
	N	80	80	80	80	80
PDW	Pearson Correlation	-.344**	-.054	.094	1	.042
	Sig. (2-tailed)	.002	.632	.406		.713
	N	80	80	80	80	80
PCT	Pearson Correlation	.213	.759**	.383**	.042	1
	Sig. (2-tailed)	.058	.000	.000	.713	
	N	80	80	80	80	80

**. Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

Chapter V

5. Discussion, Conclusions and Recommendations

5.1 Discussion

This case-control study involved 80 Sudanese pregnant women (40 induced hypertention and 40 normal pregnant) in Khartoum State .

The study showed that the mean value of Antithrombin III level , PLT Count and indices in induced hypertention pregnant women was significant value when compared to control group of normal pregnancy .

The present study found induced hypertention pregnant women had a deficient level of Antithrombin III .This finding agree with **B. A. RAMALAKSHMI *et al*,1995** in India, that found induced hypertention pregnant women had low Antithrombin III level. Also agree with Purnima Dey Sarkar, **Sonal Sogani 2013** in India ,when compared with control groups (healthy non pregnant and normal pregnant women), the levels of AT III in preeclamptic group was significantly lower.

Present study found induced hypertention pregnant women had a significant decrease of PLT Count .This finding agree with Onuigwe F. U *et al*,2015 in Nigeria, that found induced hypertention pregnant women is significantly associated with low platelet count .also agree with **Mohamed ,F.A.N (2016)** in Sudan found a highly significant lower of PLT count in Pregnant with Preeclampsia compared to normal pregnant.

Present study found induced hypertention pregnant women had a significant increased of MPV and PDW ,while a significant decrease of PCT observed . This finding agree with Nooh, **A.M. *et al*,2015** in Egypt, that found induced hypertention pregnant women observe that Platelet count (PC) was decreasing while mean platelet volume (MPV) and platelet distribution width (PDW) were increasing as PE progressed . also the study agree with **Mohamed ,F.A.N (2016)** in Sudan found Mean platelet volume and platelet width distribution were significantly higher in women with Preeclampsia compared to normal pregnant. also **Eman *et al* ., 2013**.in Egypt found that Mean platelet volume and platelet width distribution were significantly higher in women with PE compared to women with normal pregnant women groups.

In the present study the Antithrombin III , PLT Count , MPV , PDW and PCT was not affected by age .

The present study Show that there is moderate positive correlation between AT III and PLT count , week negative correlation between PLT and MPV has been found ,a week negative correlation between PLT and PDW ,a strong positive correlation between PLT and PCT , a week negative correlation between AT III and MPV , week negative correlation between AT III and PDW , a week positive correlation between AT III and PCT.

The present study also determined women with contraceptive drugs had not affected level of Antithrombin III .

The present study also determined women with contraceptive drugs had not affected PLT , MPV , PDW and PCT . This result disagree Petersen *et al*, (1978) study in Kansas, which indicated contraceptive administrator had Antithrombin111 deficiency.

5.2 Conclusions

On the bases of outcome we conclude :

- There was significant decrease in the level of AT III in pregnant women induced hypertention in compare with AT III level in normal pregnant women .
- There is a significant decrease of PLT Count of induced hypertention pregnant women in compare with PLT Count of normal pregnant women .
- There is a significant increased of MPV of induced hypertention pregnant women in compare with MPV of normal pregnant women .
- There is a significant increased of PDW of induced hypertention pregnant women in compare with PDW Count of normal pregnant women .
- There is a significant decrease of PCT of induced hypertention pregnant women in compare with PCT of normal pregnant women .
- There was no correlation between AT III level , PLT Count , MPV , PDW and PCT with age.
- There was no correlation between AT III level, MPV , PDW , PCT and PLT Count with contraceptive.

5.3 Recommendations

We recommended that:-

- Use more sensitive techniques to detect level of Antithrombin111.
- Use techniques to detect both level and activity of Antithrombin111.
- Increase the numbers of areas and collect larger amount of blood samples.
- Use PCR to detect genetic abnormality mutation in Antithrombin111.
- Platelet indices are easily obtained together with the CBC report; thus, clinicians should evaluate platelet count and indices in induced hypertention pregnant women .
- More researches should be performed for monitoring the complete coagulation profile.

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Appendix (1)

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Department of Hematology

Estimation of AntithrombinIII Level , PLT & PLT Indices in Pregnant Women Induced Hypertention

قياس معدل مضاد الثرومبين٣ ,عدد الصفائح الدموية و مؤشرات الصفائح الدموية في دم النساء الحوامل السودانيات المصابات بإرتفاع ضغط الدم

Questionnaire

Name : …….…….

ID code : ……..…… **Age :** ……

History of Contraceptive?

Yes (……..…) No (……)

Blood Pressure :

Normal (……..)

hypertension (……..)

Hypotention (……..…)

Medical condition ……

Medication Use ……..

PLT Count : ……

PLT Indices

MPV ……. (fl) PDW…… (fl)

PCT …….. (%) IPF …… (%)

Antithrombin III Leve ……..

Appendix (2)

جامعة السودان للعلوم والتكنولوجيا

كلية علوم المختبرات الطبية

مشروع مقارنة مستوى مضاد الثرومبين٣، عدد الصفائح الدموية و مؤشرات الصفائح الدموية لدى النساء الحوامل السودانيات المصابات بإرتفاع ضغط الدم في ولاية الخرطوم .

استمارة الموافقة على المشاركة فى بحث عن مقارنة مستوى مضاد الثرومبين ٣، عدد الصفائح الدموية و مؤشرات الصفائح الدموية لدى النساء الحوامل السودانيات المصابات بإرتفاع ضغط الدم في ولاية الخرطوم .

نحن اطباء وباحثون من كلية المختبرات الطبية بجامعة السودان نقوم بمسح في بعض المناطق السودانيه لوضع الاستراتيجيات المستقبليه لمواجهة لهذه المشكله.

وحتى نستطيع ان نقوم بهذا الدور نود أن نطلب منك ان تشارك فى هذا البحث. واذا وافقت سيقوم الطبيب او الكادر الطبى المدرب بأخذ عينة دم لاجراء الفحوصات .

واخذ هذة العينات لن يعرضك للخطر وسنستعمل ادواة معقمه لاخذ تلك العينات. ونعلمك بان عدم موافقتك على المشاركة فى البحث اوعدم موافقتك لن يحرمك من حقك فى العلاج والرعاية الطبية المطلوبة.

ونفيدك بانك لن تتلقى اى عائد مادى لمشاركتك فى البحث وستكون مشاركتك طوعية.

نود اخطارك بان المعلومات الخاصة بك ستكون سرية ولن يطلع عليها الاخرون ماعدى الطبيب الذى يتولى علاجك ورؤساء الفريق البحثى.

نود موافقتك على المشاركة فى هذا البحث ويمكنك أن تسال وتستفسر عن البحث ومشاركتك فيه من الاطباء والفريق الطبى المشرف على البحث قبل موافقتك النهائية ونرجو اثبات موافقتك بامضاء الاستمارة المرفقة بعد قرائتها .

للبالغين (من 18 عام)

أنا.....اؤوكد فهمى لمحتوى الاستمارة وافيد بموافقتى على المشاركة فى البحث واعلم أنه من حقى رفض المشاركة و الانسحاب من المشاركة فى اى وقت ولن يؤثر ذلك فى حقوقى .

واوافق على اخذ العينه المطلوبة للبحث.

الامضاء.....

الشاهد:.....

Appendix (3)

ACCENT-200

Appendix (4)

sysmex XP-300