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Determination of Oral Contraceptive pills Effect on Coagulation Profile among Sudanese women in Shendi locality

A thesis submitted in partial fulfillment for the requirement of MSc degree in Medical laboratory science(inHematology)

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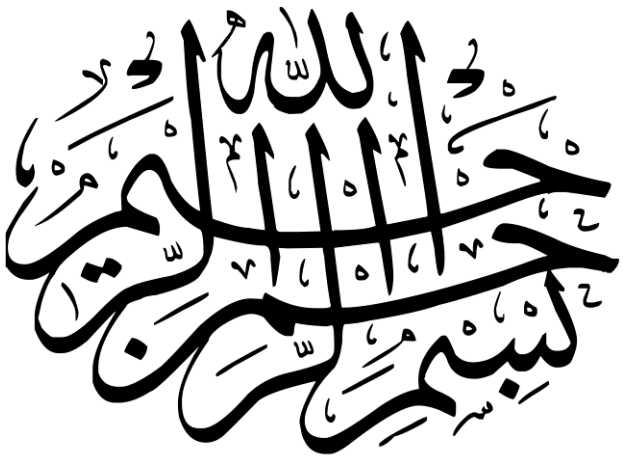
بسم الله الرحمن الرحيم

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(قَالُواْ سُبْحَانَكَ لاَعلْمَ لَنَا إِلّا مَاعَلّمْتَنَا إِنَكَ
أَنتَ الْعَلِيمُ الحَكِيمُ)

صدق الله العظيم
سورة البقرة-الآية (32)

الإهداء

إلى روح والدي العزيز رحمه الله

إلى أمي متعها الله بالصحة والعافية

فلولاهما ماوجدت في هذه الحياة ومنهما تعلمت الصمود مهما كانت الصعوبات.

إلى أساتذتي الكرام ـ الدين أضاءوا بعلمهم عقول غيرهم وهدو بالجواب الصحيح حيرة السائلين فأظهرو بسماحتهم تواضع العلماء ورحابة العارفين.

إلى إخوتي ..... وإلى من هو أقرب من روحي إليّ،

إلى الزملاء والزميلات الذين كانو خير معين في تحمل الصعاب

أهدي إليكم هدا الجهد المتواضع داعية المولى –سبحانه وتعالى ـ أن تكلل بالنجاح والقبول من جانب أعضاء لجنة المناقشة المبجلين.

شكر وتقدير

بسم الله الرحمن الرحيم

(رَبِّ أَوزِعني أَن أَشكُرَ نعمتَكَ الّتي أنَعَمتَ عَلَيّ وعَلَى والَدَيّ وأَن أَعمَلَ صَالحاً تَرضَاهُ وأَصلِح لي في ذُريّتيّ إني تُبتُ إليَكَ وإنَي منَ الْمُسلمينَ)

صدق الله العظيم

(سورة الأحقاف..الآية 15)

بعد الحمد لله والثناء عليه والصلاة والسلام على رسول الله المبعوث رحمة للعالمين

لابد لنا ونحن نخطو في سلم المعرفة والعلم أن نقف إجلالا وتعظيما لأساتذتنا الكرام الذين قدموا لنا الكثير باذلين في ذلك جهودا كبيرة .

وأخص بالشكر الجزيل الدكتورة / أم كلثوم عثمان التي رعت بتوجيهاتها وإرشاداتها وأفكارها هذا البحث .

والشكر لكل من ساعد على إتمام هدا البحث ومد لي يد العون والمساعدة وزودني بالمعلومات اللازمة .

List of abbreviations

APTT	A ctivated partial thromboplasitin time
COC	Combined Oral Contraceptive
CVD	Cardiovascular disease
OCP	Oral contraceptive pills
POP	Progestin Only Pills
PT	Prothrombin Time
Plt	Platelet
P P P	Platelet poor plasma

Abstract

This is a descriptive cross-sectional study was conducted in Shendi locality in the period from April 2018 to July 2018 to evaluate the effect of contraceptive pills on coagulation tests (Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and total count of platelets. eighty women were selected as volunteers according to inclusion criteria and considered as case, and other forty women not taken these pills, were selected and considered as control group. 4.5 ml of fresh venous blood were collected from each volunteer, after filling the questionnaire, in glass container containing 0.5 ml of 3.8% trisodium citrate solution for anticoagulation mix and immediately count the platelet using hematology analyzer. Then the contents of the container were mixed and centrifuged at 3000 round/min for 15 minutes for preparation of platelets poor plasma (PPP). The PPP were tested for the PT and APTT by using the coagulometer instrument (Clot). The results were analyzed by independent T test of the SPSS computer program.

The results of cases showed that APTT mean= 33.4 seconds, PT mean= 14.0 seconds and the mean total count of platelet =319 . when compared with results of control group revealed that APTT mean= 30.7 seconds, PT mean= 13.4 seconds and mean of platelet count=272 . Over all the results were showed significant increased in APTT when compared with control group with P value < 0.05, no significant variations was noticed in PT and with P value > 0.05).and significant higher in count of platelet($P<0.005$). and this indicate the hypercoagulability. significant changes were noticed between age groups, type of oral contraceptives and duration of uses .

Conclusion: This study concludes that OCP users had more tendency of hypercoagulability and therefore these women are at higher risk of thromboembolic effects.

الخلاصة

أجريت هذه الدراسة للكشف عن تأثير موانع الحمل الفموية في زمن البروثرومبين،زمن الثرومبوبلاستين الجزئي وعدد الصفائح الدموية في 80 مستخدمة لها بالمقارنة مع 40 من اللاتي لايستخدمن هذه الحبوب.تم سحب5.4مل من الدم الوريدي من المتبرعات –بعد الإجابة عن الإستبيان المعد مسبقاً–في حاويات زجاجية تحتوي علي 5.0 مل من38%سترات الصوديوم الثلاثية كمانع للتجلطزمجت العينة جيداً ونحسب الصفائح الدموية فوراً ثم نفصل العينة بواسطة جهاز الطرد المركزي في سرعة000.3 |الدقيقةلمدة 15 دقيقة لتحضير P.P.Pونجري إختبار البروثرومبين،زمن الثرومبوبلاستين الجزئي.أشارت النتائج إلى أن متوسط زمن الثرومبوبلاستين الجزئي=4.33|ث،ومتوسط البروثرومبين0.14=|ث ومتوسط عددالصفائح الدموية =319 وعند مقارنها مع نتائج غير المستخدمين حيث كانت نتائجهم كالآتي:متوسط زمن الثرومبوبلاستين الجزئي=7.30|ث ومتوسط البروثرومبين=4.13|ث ومتوسط الصفائح=272،تشير هذه النتائج إلى أن إستعمال موانع الحمل الفموية تؤثر في زمن الثرومبوبلاستين الجزئي وعدد الصفائح الدموية ($P<0.05$) ويزيد هذا التأثير عند الإستخدام لفترة طويلة وعند تقدم العمر وكذلك عند إستخدام الحبوب ثنائية الهرمون ،حيث حدث قصر ذو دلالة في زمن البروثرمبين وزمن الثرومبوبلاستين الجزئي وإرتفاع ذو دلالة في عدد الصفائح الدموية ،وهذا يشير إلى حالة التخثر المفرط.

توصلت هذه الدراسة إلى أن النساء الاتي يستخمن موانع الحمل الفموية أكثر عرضة لإرتفاع عوامل التجلط وبالتالي أكثر خطورة لحدوث الجلطات.

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Chapter One

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1.1. Introduction

Contraception is the use of various devices, drug agents, sexual practices or surgical procedures to prevent conception or impregnation (pregnancy). This process help couples plan when they want to have a child. The first available preparation of hormonal contraceptives contained a high dose of the estrogen EE2 which was linked to increased risk of thrombosis. Estrogen containing contraceptives particularly at a reduced dose can led to an additional risk reduction of venous thrombosis .(1)

Hormonal contraceptives are often associated with side effects commonly; nausea, headache, breast tenderness, weight gain, irregular bleeding, and mood changes[2]. Oral pills are the most frequently used hormonal contraceptives and commonly contribute to increased blood pressure, blood clots, heart attack and stroke. [3,4].

In Europe and North America studies have demonstrated that estrogen /progestogen oral contraceptives are associated with myocardial infarction, thromboembolism and stroke commonly among women over the age of 35 and smokers [5].

The lowering of the estrogen dose from >50 μg to 30 μg has been shown to be associated with a significant decrease in the risk of venous thrombosis [6].

The cause of differences in the coagulation and haemostatic status between women using hormonal contraceptives from widely diverse geographical areas is not clearly understood.

WHO recommended that studies should be conducted in different settings to bring about a clearer picture [7].

The most drastic adverse effect associated with hormonal contraceptive use is predisposition to higher risk of thromboembolic phenomena. Progestins have

antiplasmin and antithrombin activity Its use increase platelet count and aggregability, thus predisposing to hypercoagulability. [8].

A previous study by. Naess IA, Christiansen SC, Romundstad P, Cannegieter SC, Rosendaal FR, ct al. (2007) , reported an increased rate of thrombosis of 1-3 per 100,000 individuals per year . (9).

1.2. Justification

Hormonal contraceptives are widely used, and contain different doses of estrogen and types of progestogen. However, these hormonal contraceptives may potentially be associated with some side effects of coagulopathy.

Epidemiologic studies were found a relationship between oral contraceptive use and altered level of coagulation factors, platelet changes and thromboembolic phenomenon.

OCP users developed a state of hypercoagulability that was indicated by the significant elevation of plasma fibrinogen level, factor XII, vitamin K dependent clotting factors, f actor VII and total count of platelets which can lead to thromboembolic episodes .

Higher level of blood coagulation factors and activated protein C resistance were the causative factors for the risk of development of venous thromboembolism in women taking OCP .

So this study was conducted to determine of Oral Contraceptive pills Effect on Coagulation Profile among Sudanese women in shendi locality, with aim of identify its magnitude and consequently altering obstetricians in order to properly asses their patients who are on the pills

so that they can decide whether to continue taking them or not ,in order to prevent unnecessary thrombotic incidence.

1.3. Objectives

General objective:

Determination of contraceptive pills effect on coagulation profile among Sudanese women in Shendi locality .

Specific objectives:

1- To determine platelet count.

2- To estimate prothrombin Time (PT).

3- To estimate activated partial thromboplastin time (APTT).

4- To compare the result of these tests between case and control.

5- To compare the result of these tests between different age groups.

6- To determine the effect of duration of uses of pills on coagulation tests.

7- To determine the effect of different types of oral contraceptives on coagulation tests.

Chapter Two

literature review

2.1. Contraception

The combined formulation of estrogen and progestin are widely used as temporary contraceptive. However this safe use has been remained under debate .(10).

Combined hormonal oral contraceptives containing less than 50ug ethinyl estradiol has been named as low dose oral contraceptives.(11).

Despite the the general acceptability and the obvious advantages that have been attributed to low dose oral contraceptive use ,some serious side effects have been reported in women taking the pills.

Epidemiologic studies were found a relationship between oral contraceptive use and altered level of coagulation factor ,platelet changes and thromboembolic phenomenon.(12).

O.C.P users developed estate of hyper coagulability that was indicated by the significant elevation of plasma fibrinogen level, factor XII ,vitamin K dependent clotting factors and total count of platelets which can lead to thromboembolic episodes.(13) (14) (15).

There are several type of contraception methods involving different mechanism :

1. Natural methods.
2. Injectables.
3. Implants.
4. Spermicides.
5. Condom.
6. Diaphragm.
7. Postcoital Douching.
8. Postcoital contraception.
9. Sterilization.
10. Contraceptive pills.

2.1.1 The action of hormonal method

The action of agents affects three aspect:

- The suppression of ovulation.
- Disruption of cervical mucous which be curses ,thicker so that is difficult for sperm to travel .
- Changes the endometrium implantation mor difficult thus causing loss of zygote.

These effects occur due to action on the hypothalamus and for the pituitary gland in the brain which produce hormones which affect the cycle .some of the preparation work by suppressing follicle stimulating hormone (FSH) and for luteinizing hormone (LH) and thussuppres development and ovulation.[10] [11].

2.1.2 Oral Contraceptive Pills

Oral contraceptives are widely used, and their usage and risk of developing cancer and cardiovascular disease for certain groups of women has been the subject of many studies.[10].

Oral contraceptives first become available to American women in the early 1960.popular known as the pills, it was the first generation which contained high dose of progestin and estrogen. second generation progestin, was developed in 1970.seven years ago ,third generation become available to reduce the androgenic and metabolic side effects that occur with older agents.[11].

2.1.3 Types of oral contraceptive pills

There are two types of oral contraceptive pills:

1. Two man-made versions of natural females hormones(estrogen and progestin) known as combined pills. that are similar to the hormones of the ovaries which are normally produced -Estrogen stimulates the growth and the development of

the uterus at puberty ,causes the endometrium thicken during the first half of menstrual cycle ,and cause change in breast tissue at puberty and at child birth.

- Progestin, which is produced during the last half of the menstrual cycle ,prepare the endometrium to receive the egg, if egg is fertilized progesterone secretion continues preventing release of additional eggs from the ovaries for this reason, progesterone is called the pregnancy supporting hormone and scientists believe that it has valuable contraceptive effects.

2- Second type: is called mini pills, it contains only a man made progesterone used in oral contraception called progesogen or progestin .the mini pills are less effective in preventing pregnancy than the combination pills.[12].

2.1.4 Oral contraceptive : benefits and risk:-

Oral contraceptive like any other effective medicine ,have undesired side effects .in rare cases headache, gastric upsets, nausea ,feeling of tension in breast, chang in body weight and allergic skin rash..but they have beneficial effects :they reduce the risk of cancer of the ovary and reduce incidence of benign brest disease.[13].

- The most important cardiovascular complication in oral contraceptive users are: thromboembolism – myocardial infraction and thrombotic strokes with higher risk and susceptibility in female smokers in the age range.these are attributed to estrogenic component, and were found to be reduced after the introduction of oral contraceptive containing a lower dose of estrogen.[14].
- Venous thromboembolism including:-pulmonary and deep venous thrombosis is the most common serious cardiovascular event among women who use oral contraceptives.

Women who are taking pills have three to six time's greater risk of venous thromboembolism than women who don't use this contraceptive method and risk increase with age, obesity and recent surgery.

The pills may influence the effect on coagulation and fibrinolytic markers as well as on lipid metabolism.[14].

The effect on haemostasis is an increase in the level of some factors(factor I I -VII –IX –X –XI and VIII)VW - fibrinogen- protein C and complexes and fragments related to activation of coagulation(thrombin –anti thrombin complex and D-dimer),these enhance fibrinolysis and decrease level of anti thrombin III and protein .

2.2. Normal Haemostasis

The haemostatic mechanisms have several important functions**:**

(1) To maintain blood in a fluid state while it remains circulating within the vascular system.

(2) To arrest bleeding at the site of injury or blood loss by formation of a haemostatic plug.

(3) To limit this process to the vicinity of the damage.

(4) To ensure the eventual removal of the plug when healing is complete.

Normal physiology thus constitutes a delicate balance between these conflicting tendencies and a deficiency or exaggeration of any one may lead to either thrombosis or haemorrhage. There

are at least five different components involved: blood vessels, platelets, plasma coagulation factors and their inhibitors and the fibrinolytic system.

The normal haemostatic response to vascular damage depends on a closely linked interaction between the blood vessel wall, circulating platelets and blood coagulation factors .

An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is clearly essential for survival.

Nevertheless, such a response needs to be tightly controlled to prevent extensive clots developing and to be able to break down such clots once damage is repaired.

The haemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process for fibrinolysis.[15].

2.2.1 Components of normal homeostasis

2.2.1.1. Blood Vessels

General Structure of the Blood Vessel

The blood vessel wall has three layers: intima, media and adventitia. The intima consists of endothelium and subendothelial connective tissue and is separated from the media by the elastic lamina interna. Endothelial cells form a continuous monolayer lining all blood vessels.

The structure and the function of the endothelial cells vary according to their location in the vascular tree, but in their resting state they all share three important characteristics:

they are 'non-thrombogenic' (i.e. they promote maintenance of blood in its fluid state); they play an active role in supplying nutrients to the subendothelial structures, and they act as a barrier to cells, macromolecules and particulate matter circulating in the bloodstream. The permeability of the endothelium may vary under different conditions to allow various molecules and cells to pass.(16).

The function of endothelial cells:

- Produce von willebrand factor for platelet adhesion to sub endothelial layer.

- Produce prostaglandin and nitric oxide which have vasodilatory properties and inhibit platelets aggregation .
- Produce thrombomodulin which act a proteins receptor.
- Produce tissue-plasminogen activator to active fibrinolysis.
- Contain heparin sulphate and other glycols aminoglycan which are capable of activating anti thrombin .
- -Synthesize protein S.

The components of sub endothelial cell are exposed after damaged and are responsible for platelets adherence to collagen, which mediated by von wille brand factor.

Vasoconstriction

Vessels with muscular coats contract following injury, thus helping to arrest blood loss. Although not all coagulation

reactions are enhanced by reduced flow, this probably

assists in the formation of a stable fibrin plug by allowing activated factors to accumulate to critical concentrations.

Vasoconstriction also occurs in the microcirculation in vessels without smooth muscle cells. Endothelial cells themselves can produce vasoconstrictors such as angiotensin II. In addition, activated platelets produce thromboxane A2 (TXA2), which is a potent vasoconstrictor. (16).

2.2.1.2 Platelets

Platelet production: Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte – the megakaryoblast– arises by a process of differentiation from the haemopoietic stem cell . The megakaryocyte matures by endomitotic synchronous replication (i.e. DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increases in multiples of two.

Early on invaginations of plasma membrane are seen, called the demarcation membrane, which evolves through the development of the megakaryocyte into a highly branched network. At a variable stage in development the cytoplasm becomes granular. Mature megakaryocytes are extremely large, with an eccentrically placed single lobulated nucleus and a low nuclear : cytoplasmic ratio .

Platelets form by fragmentation from the tips of cytoplasmic extensions of megakaryocyte cytoplasm, each megakaryocyte giving rise approximately to 1000–5000 platelets . The platelets are released through the endothelium of the vascular niches of the marrow where megakaryocytes reside. The time interval from differentiation of the human stem cell to the production of platelets averages 10 days.

Thrombopoietin (TPO) is the major regulator of platelet formation and 95% is produced by the liver. Approximately 50% is produced constitutively, the plasma level depending on its removal from plasma by binding to c-MPL receptors on platelets and megakaryocytes . Therefore, levels are high in thrombocytopenia as a result of marrow a plasia but low in patients with raised platelet counts. The other 50% is regulated in response to platelet destruction. As platelets age they lose surface sialic acid. This exposes galactose residues that attach to the Ashwell–Morell receptor in the liver. This attachment signals for production of new TPO . TPOincreases the number and rate of maturation of megakaryocytes via c-MPL receptor. Platelet levels start to rise 6 days after the start of therapy. Although TPO itself is not available for clinical use, thrombomimetic agents which bind to c-MPL are now used clinically to increase the platelet count .

The normal platelet count is approximately 250 × 109/L(range 150–400 × 109/L) and the normal platelet life span is 10days. This is determined by the ratio of the apoptotic BAX and anti-apoptotic BCL 2 proteins in the cell. Up to one-third of the marrow output of platelets may be trapped at any one time in the normal spleen but this rises to 90% in cases of massive splenomegaly.[17].

Platelet structure: Platelets are extremely small and discoid, 3.0 × 0.5 μm in diameter.The glycoproteins of the surface coat are particularly important in the platelet reactions of adhesion and aggregation, which are the initial events leading

to platelet plug formation during haemostatic. Adhesion to collagen is facilitated by glycoprotein Ia (GPIa) . Glycoproteins Ib (defective in Bernard–Soulier syndrome) and IIb/IIIa (also called αIIb and β3, defective in Glanzmanns thrombasthenia)are important in the attachment of platelets to von Willebrand factor (VWF) and hence to vascular sub endothelium . The binding site for IIb/IIIa is also the receptor for fibrinogen which, like VWF, is important in platelet–platelet aggregation.

The plasma membrane invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids (previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin(factor II) to thrombin (factor IIa) .

The platelet contains three types of storage granules: dense, α and lysosomes. The more frequent specific α granules contain clotting factors, VWF, platelet-derived growth factor (PDGF) and other proteins. Dense granules are less common and contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin and calcium. Lysosomes contain hydrolytic enzymes. Platelets are also rich in signaling and cytoskeletal proteins, which support the rapid switch from quiescence to activation that follows vessel damage. During the release reaction , the contents of the granules are discharged into the open canalicular system.[17].

Platelet antigens: Several platelet surface proteins have been found to be important antigens in platelet-specific autoimmunity and they have been termed human platelet antigens (HPA). In most cases, two different alleles exist, termed a or b alleles (e.g. HPA-1a).

Platelets also express ABO and human leucocyte antigen (HLA) class I but not class II.[17].

Platelet function: The main function of platelets is the formation of mechanical plugs during the haemostatic response to vascular injury.

In the absence of platelets, spontaneous leakage of blood

through small vessels may occur. There are three major platelet functions: adhesion, aggregation and release reactions and amplification.

The immobilization of platelets at the sites of vascular injury requires specific platelet–vessel wall (adhesion) and platelet–platelet (aggregation) interactions, both partly mediated through VWF. [17].

Von Willebrand factor (VWF)

VWF is involved in shear-dependent platelet adhesion to the vessel wall and to other platelets (aggregation) . It also carries factor VIII. It is a large glycoprotein, with multimers made up on average of 2–50 dimeric subunits. VWF is synthesized both in endothelial cells and megakaryocytes, and stored in Weibel–Palade bodies and platelet α granules, respectively.

Plasma VWF is almost entirely derived from endothelial cells, with two distinct pathways of secretion. The majority is continuously secreted and a minority is stored in Weibel–Palade bodies. The stored VWF can raise the plasma levels when released under the influence of several secretagogues, such as stress, exercise, adrenaline and infusion of desmopression(1-diamino -8-D-arginine vasopressin; DDAVP). The VWF released from Weibel–Palade bodies is in the form of large and ultra-large multimers, the most adhesive and reactive form of VWF. They are in turn cleaved in plasma to smaller multimers and monomeric VWF by the specific plasma metalloprotease,ADAMTS13 .[17].

Platelet aggregation: This is characterized by cross-linking of platelets through active GPIIb/IIIa receptors with fibrinogen bridges. A resting platelet has GPIIb/IIIa receptors which do not bind fibrinogen, VWF or other ligands. Stimulation of a platelet leads to an increase GPIIb/IIIa molecules, enabling platelet cross-linking via VWFand fibrinogen bridges.[17].

Platelet reactions and primary haemostatic plug Formation

Following a break in the endothelial lining, there is an initial adherence of platelets (via GP1a and GP1b receptors) to -exposed connective tissue, mediated (GP1b) by VWF. Under conditions of high shear stress (e.g. arterioles) the exposed subendothelial matrix is initially coated with VWF. Collagen exposure and thrombin generated through activation of tissue factor produced at the site of injury cause the adherent platelets to release their granule contents and also activate platelet prostaglandin synthesis, leading to the formation of TXA2. Released ADP causes platelets to swell and aggregate.

Platelet rolling in the direction of blood flow over exposed VWF with activation of GPIIb/IIIa receptors results in firmer binding. Additional platelets from the circulating blood are drawn to the area of injury. This continuing platelet aggregation promotes the growth of the haemostatic plug, which soon covers the exposed connective tissue. The unstable primary haemostatic plug produced by these platelet reactions in the first minute or so following injury is usually sufficient to provide temporary control of bleeding. The highly localized enhancement of platelet activation by ADP and TXA2 results in a platelet mass large enough to plug the area of endothelial injury. [17].

Platelet procoagulant activity: After platelet aggregation and release, the exposed membrane phospholipid (platelet factor 3) is available for two reactions in the coagulation cascade. Both phospholipid□ mediated reactions are calcium□ ion

dependent. The first (tenase) involves factors IXa, VIIIa and X in the formation of factor Xa (Fig. 24.8). The second (prothrombinase) results in the formation of thrombin from the interaction of factors Xa, Va and prothrombin (II).

The phospholipid surface forms an ideal template for the crucial concentration and orientation of these proteins.

Growth factor:PDGF found in the α granules of platelets stimulates vascular smooth muscle cells to multiply and this may hasten vascular healing following injury.

Natural inhibitors of platelet function: Nitric oxide (NO) is constitutively released from endothelial cells (Fig. 24.9) and also from macrophages and platelets. It inhibits platelet activation and promotes vasodilatation. Prostacyclin synthesized by endothelial cells also inhibits platelet function and causes vasodilatation by raising cyclic guanosine monophosphate (GMP) levels. An ectonucleotidase (CD39) acts as an ADPase and helps prevent platelet aggregation in the intact vessel wall.

Never the less, because of incorporation of plasminogen and TPA, this plug begins to autodigest during the same time frame. (17).

2.2.1.3 Blood coagulation

The coagulation cascade

Blood coagulation in vivo involves a biological amplification system in which relatively few initiation substances sequentially activate by proteolysis a cascade of circulating precursor proteins(the coagulation factor enzymes) which culminates in the generation of thrombin; this, in turn, converts soluble plasma fibrinogen into fibrin . Fibrin enmeshes the platelet aggregates at the sites of vascular injury and converts the unstable primary platelet plugs to firm, definitive and stable haemostatic plugs.

The operation of this enzyme cascade requires local concentration of circulating coagulation factors at the site of injury. Surface-mediated reactions occur on exposed collagen, platelet phospholipid and tissue factor. With the exception of fibrinogen, which is the fibrin clot subunit, the coagulation factors are either enzyme precursors or cofactors . All the enzymes, except factor XIII, are serine proteases (i.e. their XI through sequential activation of factors IX, X and prothrombin may generate up to 2 × 108 mol of fibrin) [17].

Coagulation invivo: The generation of thrombin in vivo is a complex network of amplification and negative feedback loops to ensure a localized and limited production. The generation of thrombin isdependent on three enzyme complexes, each consisting of protease, cofactor, phospholipids (PL) and calcium. They are:

(i) extrinsic Xase (VIIa, TF, PL, Ca2+) generating FXa;

(ii) intrinsic Xase (IXa, VIIIa, PL, Ca2+) also generating FXa;

(iii) prothrombinase complex (Xa, Va, PL, Ca2+) generating thrombin.

The generation of thrombin following vascular

injury occurs in two waves of very different magnitude.

During the initial phase small amounts of thrombin are generated (picomolar concentrations). This thrombin leads to a second million times larger burst of thrombin production.[17].

Initiation: Coagulation is initiated after vascular injury by the interaction of the membrane bound tissue factor (TF), exposed and activated by vascular injury, with plasma factor VII.

It is expressed on fibroblasts and small muscles of the vessel wall and in the blood stream on microparticles, and on other non-vascular cells. The factor VIIa–tissue factor (extrinsic factor Xase) complex activates both factor IX and factor X.

The factor Xa, in the absence of its cofactor, forms small

amounts of thrombin from prothrombin. This is insufficient to initiate significant fibrin polymerization. Amplification is needed. [17]

· **Amplification:** The initiation pathway or extrinsic Xase is rapidly inactivated by tissue factor pathway inhibitor (TFPI). Thrombin generation is now dependent on the traditional intrinsic pathway In this factor VIII and V are converted

The intrinsic (contact), extrinsic and common pathways of blood coagulation. The APPT tests the intrinsic and common pathways, the PT the extrinsic and common pathways and the TT tests for thrombin inhibitors and deficiency or abnormality of fibrinogen. HMWK, high molecular weight kininogen; APPT, PT, TT, to VIIIa and Va by the small amounts of thrombin generated

during initiation. In this amplification phase the intrinsic Xase, formed by IXa and VIIIa on phospholipid surface in the presence of Ca2+, activates sufficient Xa, which then, in combination with Va, PL and Ca2+, forms the prothrombinase complex and results in the explosive generation of thrombin which acts on fibrinogen to form the fibrin clot.

Factor XI does not seem to have a role in the physiological initiation of coagulation. It has a supplementary role in the activation of factor IX (see above) and may be important at major sites of trauma or at operations, in which situations factor XI deficient individuals tend to bleed excessively. It is also involved in the contact pathway .

Thrombin hydrolyses fibrinogen, releasing fibrinopeptides A and B to form fibrin monomers .monomers link spontaneously by hydrogen bonds to form a loose insoluble fibrin polymer. Factor XIII is also activated by thrombin and stabilizes the fibrin polymers with the formation of covalent bond cross - links.

Fibrinogen consists of two identical subunits, each containing three dissimilar polypeptide chains (α, β and γ) which are linked by disulphide bonds. After

cleavage by thrombin of small fibrinopeptides A and B from the α and β chains, fibrin monomer consists of three paired α, β and γ chains which rapidly polymerise.

The activity of factors II, VII, IX and X is dependent upon vitamin K, which is responsible for carboxylation

of a number of terminal glutamic acid residues on each of these molecules .

Although factor VIII and V cofactors are not protease enzymes, they circulate in a precursor form that requires limited cleavage by thrombin for expression of full cofactor.[17].

Table (2-1): coagulation factor

The co Factor Number	Descriptive name	Active Form
I	Fibrinogen	Fibrinsubunit
II	Prothrombin	Serineprotease
III	Tissue factor	Cofactor
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart–Prower factor	Serine protease
XI	Plasmathromboplastin antecedent	Serine protease
XII	Hageman factor	Serine protease
XIII	Fibrin-stabilizing factor	Transglutamin
Prekallikrein	Prekallikrein	Serine protease
HMWK	Fitzgerald factor	Cofactor

aPhysiological limitation of blood coagulation blood coagulation would lead to dangerous occlusion of blood vessels (thrombosis) if the protective mechanisms of coagulation factor inhibitors, blood flow and fibrinolysis were not in operation.(17).

Clotting mechanism begins by Trauma to tissues or trauma to blood.In each case it leads to formation of prothrombin activator which causes conversion of prothrombin in to thrombin. There are two pathways of formation of prothrombin activator:

i) Extrinsic Pathway.

ii) Intrinsic pathway.

It begins with trauma to Vascular wall or to the tissues blood itself outside the blood vessel. In both pathways, different blood clotting factors play important roles.

Davie and Ratnoff (1965) have proposed a waterfall sequence hypothesis to explain the events taking place in coagulation process. Where as Macfarlane has suggested a scheme of coagulation called enzyme cascade which is similar to waterfall sequence.

Blood clotting factors exist in inactive form and are activated sequentially until finally prothrombin activator is formed.

Extrinsic Mechanism:

(Factors involved – III-VII-X-V) for formation of prothrombin activator .

1) It begins with trauma to blood vessel or tissues outside the blood vessel. It releases tissue factor and Tissue phospholipids and clotting process starts.
2) The tissue factor complexes with blood clotting factor VII and activates it.
3) Activated factor VII in presence of ca++ and tissue phospholipids acts on factor –X and activates it.

4) Activated factor X acts on Factor V and activates it.

5) Activated F-X complexes with tissue phospholipids, Factor-V, ca++ And forms a complex called prothrombin activator.

6) Prothrombin activator converts prothrombin in to thrombin under influence of ca++

7) Thrombin acts on fibrinogen and converts it in to fibrin monomers

8) Fibrin monomers polymerize with other fibrin monomers and form long fibrin threads that form reticulum of the clot.

9) At first clot is weak but later on with the help of active fibrin stabilizing factor (F- X III) clot becomes strong.

10) WBCs and RBCs get trapped in to reticulum of the clot

11) Clots adhere to the damaged surface of the blood vessel and thereby prevents the blood loss.

12) Clot retraction Following clot formation, the volume of the clot decreases , this is called as clot retraction platelets are necessary for clot retraction.contain contractile protein Thrombosthenin, which contracts and reduces the volume of the clot. Following this a clear fluid is separated out called as serum. [15].

Extrinsic Pathway of Blood Coagulation :

Truama to blood vessel/ Tissue rupture

↓

Tissue Factor and Tissue Phospolipids

Tissue Factor(F-III) + Factor VII (Proconvertin) → Activated Factor VII (Proconvertin/Stable Factor)

↙ ca++

Acts on Factor X(Stuart Factor) → Activated Factor X

↙ ca++

Acts on Factor V(Proaccelerin/labile Factor) → Activated Factor V

↙ ca++

Activated Factor X+ Activated Factor V + ca++ + Tissue Phospolipids

↓

Form a complex Prothrombin Activator

↓ ca++

Prothrombin (F-II) → Thrombin

↙ ca++

Fibrinogen(F-I) → Fibrin monomers

↙ ca++

Fibrin monomers → Polymerization

↓ Fibrin stabilizing factor- F-XIII

Reticulum + RBC& WBC →clot.

Intrinsic Mechanism:

(F III- V- VIII- IX –X –XI –XII)

begins with injury to blood itself and continues through following steps :

1. Trauma to blood alters two important clotting factors in the blood Factor XII and Platelet Phospholipids i.e. F- III.
2. When F-XII comes in contact with collagen outside the blood vessel, it gets activated and acts as an enzyme for activation of F-XI.
3. Damaged platelets adhere to the wet surface of blood vessel and release platelet phospholipids i.e. F- III.
4. Activated factor XII acts enzymatically on F-XI i.e. Plasma Thromboplastin Antecedent (PTA –Factor) and activates it.
5. Activated factor XI acts enzymatically on F- IX i.e. Christmas factor and activates it (ca++ are necessary) .
6. Factor IX activates F-VIII (Anti Haemophilic Factor).
7. activated F- IX , F-VIII and platelet phospholipids, activate factor-X .
8. Activated Factor X acts enzymatically on Factor V (Proaccelarin) and activates it, (ca++ are necessary).
9. Activated F-V, activated X ,Platelet phospholipids and ca++ form a complex called prothrombin activator Prothrombin activator converts prothrombin in to thrombin under influence of ca++ .
10. Thrombin acts on fibrinogen and converts it in to fibrin monomers .
11. Fibrin monomers polymerize with other fibrin monomers and form long fibrin threads that form reticulum of the clot.
12. At first clot is weak but later on with the help of active fibrin stabilizing factor (F- X III) clot becomes strong.
13. WBCs and RBCs get trapped in to reticulum of the clot

14. Clots adhere to the damaged surface of the blood vessel and thereby prevents the blood loss.

Intrinsic pathway of clotting mechanism

Injury to blood or trauma to blood

Blood comes in contact with collagen outside the blood vessel, F-XII

get activated and damaged platelets release F-III i.e.platelet phospholipids

F-XII Acts on Factor XI(Plasma Thromboplastin Antecetent) → Activated Factor XI

↙ ca++

F-XI Acts on Factor IX (Christmas Factor) → Activated Factor I X

↙ ca++

F-IXActs on Factor VIII (Anti haemophilic Factor) → Activated Factor VIII

↙ ca++

Activated Factor VIII + Platelet Phospholipids + ca++ → Act on Factor X(Stuart Factor) ↙ ca++

Activated Factor X acts on Factor -V → Activated Factor V(Proaccelerin)

Activated Factor X+ Factor V + Platelet Phospholipids + ca++.

Form a complex Prothrombin Activator.

↓ ca++

Prothrombin (F-II) → Thrombin

↙ ca++

Fibrinogen(F-I) → Fibrin monomers

↙ ca++

Fibrin monomers → Polymerization

↓ Fibrin stabilizing factor- F-XIII

Reticulum + RBC& WBC →clot [17].

Fibrinogen: Fibrinogen is a large dimeric protein, each half consisting of three polypeptides named Aa, Bb and g held together by 12 disulphide bonds. The two monomers are joined together by a further three disulphide bonds.

A variant g chain denoted g0 is produced by a variation in messenger RNA splicing. In the process a platelet binding site is lost and high-affinity binding sites for FXIII and thrombin are gained. The g0 variant constitutes approximately 10% of plasma fibrinogen. A less common(<2%) g chain variant 'gE' is also produced by splice variation.

Fibrinogen is also found in platelets, but the bulk of this is derived from glycoprotein IIbIIIa-mediated endocytosis of plasma fibrinogen, which is then storedin alpha granules, rather than synthesis by megakaryocytes.

Fibrin is formed from fibrinogen by thrombin cleavage releasing the A and B peptides from fibrinogen. This results in fibrin monomers that then associate and precipitate forming a polymer that is the visible clot. The central E domain exposed by thrombin cleavage binds with a complementary region on the outer or D domain of another monomer. The monomers thus assemble into a staggered overlapping two-stranded fibril. More complex interactions subsequently lead to branched and thickened fibre formation, making a complex mesh that binds and stabilizes the primary platelet plug.[17]

2.2.1.4. Fibrinolytic System:

The deposition of fibrin and its removal are regulated by the fibrinolytic system. Although this is a complex multicomponent system with many activators and inhibitors, it centres around the fibrinogen- and fibrin-cleaving enzyme plasmin. Plasmin circulates in its inactive precursor form, plasminogen, which is activated by proteolytic cleavage. The principal plasminogen activator (PA) in humans is tissue plasminogen activator (tPA), which is another serine protease. tPA and

plasminogen are both able to bind to fibrin via the amino acid lysine. Binding to fibrin brings tPA and plasminogen into close proximity so that the rate of plasminogen activation is markedly increased and thus plasmin is generated preferentially at its site of action and not free in plasma. The second important physiological PA in humans is called urokinase (uPA).

This single chain molecule (scu-PA or pro-urokinase) is activated by plasmin or kallikrein to a two-chain derivative (tcu-PA), which is not fibrin-specific in its action. However, the extent to which this is important in vivo is not clear and the identification of cell surface receptors for uPA suggests that its primary role may be extravascular.

The contact activation system also appears to generate some plasminogen activation via factor XIIa and bradykininstimulated release of tPA. The degradation products released by the action of plasmin on fibrin are of diagnostic use and are discussed later in this chapter. The activation of plasmin on fibrin is restricted by the action of a carboxypeptidase, which removes the amino terminal lysine residues to which plasminogen carboxypeptidase is activated by thrombomodulin-bound thrombin and is referred to as thrombin-activated fibrinolysis inhibitor (TAFI).

PAI-1 (plasminogen activator inhibitor-1) is a potent inhibitor of tPA, produced by endothelial cells, hepatocytes, platelets and placenta. Levels in plasma are highly variable. It is a member of the serpin family and is active against tPA and tcu-PA. A second inhibitor PAI-2 has also been identified, originally from human placenta, but its role and importance are not yet established.

The main physiological inhibitor of plasmin in plasma is plasmin inhibitor (a2-antiplasmin), which inhibits plasmin function by forming a 1:1 complex (plasmin–antiplasmin complex, PAP). This reaction in free solution is extremely rapid but

depends on the availability of free lysine-binding sites on the plasmin. Thus, fibrin-bound plasmin in the clot is not accessible to the inhibitor. Deficiencies of the fibrinolytic system are rare but have sometimes been associated with a tendency to thrombosis or haemorrhage.

It is the body's system for dissolution of clots. In this system ,the inactive precursor plasminogen is converted to plasmin , a serine protease with specific fibrinolytic activity, this conversion occurs through the action of tissue plasminogen activator, plasmin degrades the fibrin clot.[16].

2.2.1.5. Inhibitors of coagulation factors:

A number of mechanisms exist to ensure that the production of the fibrin clot is limited to the site of injury and is not allowed to propagate indefinitely. there are a number of proteins that bind to and inactivate the enzymes of the coagulation cascade. Probably the first of these to become active is TFPI, which rapidly quenches the factor VIIa–TF complex that initiates coagulation. It does this by combining first with factor Xa, so that further propagation of coagulation is dependent on the small amount of thrombin that has been generated during initiation being sufficient to activate the intrinsic pathway.

The principal physiological inactivator of thrombin is antithrombin (AT, formerly ATIII), which belongs to the serpin group of proteins. This binds to factor IIa forming an inactive thrombin–antithrombin complex (TAT), which is subsequently cleared from the circulation by the liver. This process is greatly enhanced by the presence of heparin or vessel wall heparan. AT is responsible for approximately 60% of thrombin-inactivating capacity in the plasma, the remainder is provided by heparin cofactor II and less specific inhibitors such as a2 macroglobulin.

AT is also capable of inactivating factors X, IX, XI and XII but to lesser degrees than thrombin.

As thrombin spreads away from the area of damage it is also bound by thrombomodulin on the surface of endothelial cells. In this way it is changed from a primarily procoagulant protein to an anticoagulant one. Although remaining available for binding to AT, thrombin bound to thrombomodulin no longer cleaves fibrinogen. It now has a greatly enhanced preference for PC as a substrate.

PC is presented to the thrombin–thrombomodulin complex by the endothelial protein C receptor (EPCR) and when activated by thrombin cleavage acts to limit and arrest coagulation by inactivating factors Va and VIIIa.

This action is further enhanced by its cofactor, protein S, which does not require prior activation. The role of EPCR is particularly important in larger vessels, where the effective concentration of thrombomodulin is low. PC is subsequently inactivated by its own specific inhibitor .(16).

Natural occurring anti coagulation:

Anti thrombin III ----> serine protease -inhibit thrombin and factor Xa , IXa and XIa.

Protein C ---→ serine protease and in activates factor Va and VIIIa in presence of protein S and phospholipids .

Heparin ---→ a protease inhibitors and act on thrombin .

α2 –macro globulin inhibit thrombin and various other protease.

2.2.2 Tests of haemostatic function:

include the thrombin time (TT), prothrombin time (PT), activated partial thromboplastin time (APTT) as well as individual coagulation factor assays and assay of von Willebrand factor. Tests of platelet function include the PFA-100 and platelet aggregation tests.

In UK the National Center for Clinical Excellence (NICE) and the British committee for Standard in Hematology (BCSH) have both addressed the value of

screening test in hemeostasis .The tests that are commonly used as screening test are :-

1- Prothrombin time (PT)

2- Activated partial theromboplastin time (APTT)

The fibrinogen concentration and thrombin time are not generally considered to be first line screening tests although they are commonly performed .

Its important that the platelet count is checked in any patient undergoing haemostatic investigation.

2.2.2.1. Platelet Count:

There are three methods to count platelets:

- From stained film:

  Examine platelets on the blood smear for number and morphology .it should show approximately 8-20 platelet per field .using 5-10 different field and multiplies this result by 20,000 to option approximation of platelet count. [17].

  Platelets count = number of platelets in fields/ number of field ×20,000.

- Automated counter:
- Is based on tow principles:

1- Impedance counting :depends on that cells are poor conductors of electricity while the blood diluents are good conductors, diluted suspension (blood and buffered electrolyte solution)is put in electrical field and change in electrical resistance that result from passage of cells through aperture are recorded.

2- Optical cells counter: passage of cell causes the light to be scattered ,these are collected by photomultiplier tube and then generalized pulses are counted .

The disadvantages of automated platelet count is that it is affected by blood sample characteristics ,and to avoid false count of microcytic hypochromic or fragmented

or giant cells, one must adjust the threshold ,automated counters produce platelets count with precision which is much superion to that of manual.[13].

- Manual technique using neubar or improved chambers:

1- Using 1% aqueous ammonium oxalate in which the red cells are lysed but is good media for bacterial contamination.

2- Using 1% formal citrate which leaves the red cells intact and is more likely to give incorrect result when the platelets count is low.[15].

Platelet = Number of platelets ×Dilution factors/Depth×Area counted.

Normal rang: 150,000-400,000 cell/ul.

-Errors in manual technique:-

- Technical errors

  -poor technique in obtaining the blood sample .

  -In sufficient mixing of the sample.

  -Inadequate mixing of the cell suspension.

  -Faulty filling of the counting chamber.

  -In accurate pipetting or use of badly calibrated pipette or counting chamber which must be free from scratches and dust practices also the cover glass which should be free from bowing and sufficiently thick so as not bend when pressed on chamber.

- Inherent errors

  Are due to uneven distribution of cells in the counting chamber .inherent errors can only be reduced by counting more cells in a preparation.[13].

2.2.2.2. Screening test

Prothrombin Time (PT)

The prothrombin time was described by Quik in 1935 and the test was often referred to as Quick's prothrombin time the prothrombin time was developed to measure prothrombin (Factorוו) and hence its name. However, it subsequently become clear that it was sensitive to abnormalities of factors vוו, x, v, וו and fibrinogen .

The prothrombin time (PT) in contrast to the APTT measures the activity of the intrinsic , so-called extrinsic and common pathways of coagulation.

The prothrombin time is a one –stage test based upon the time required for a fibrin clot to form after the addition of tissue factor (TF) (tissuethromboplastin) ,phospholipid and calcium to decalcified, platelet poor plasma.

The term Thromboplastin was originally used to describe a substance in plasma that converted prothrombin to thrombin.

Historically thromboplastin were extracted from brain and other organs and these contained significant amounts of tissue factor(TF) and phospholipid(PL).TF is species specific and most laboratories now use a recombinant human TF with an ISI.

TF was originally designated factor III when the nomenclature of the clotting proteins was undertaken.

- Principles

The PT measures the activity of the extrinsic and common pathways of coagulation and therefore ,is dependent on the functional activity of factors VII-X-V-II(prothrombin)and fibrinogen.

-Reaction involved in PT test

:VII-----TF-------->VIIa

VIIa activat factor X---------->Xa

Xa convert Prothrombin -------->Thrombin which convert fibrinogen ------>fibrin.

Reference ranges

The reference range depends upon

- Source of tissue factor e.g. human, rabbit, etc.
- The exact technique used e.g. manual or automated .
- Method of end-point determination e.g. optical or mechanical.
- Each laboratory must establish its own reference range but in general the reference range for the pro thrombin time is in range on 13-15s .

Activated Partial Thromboplastin Time (APTT)

The APTT is measure the activity of intrinsic and common pathways of coagulation. the term Activated Partial Thromboplastin Time(APTT) derives from the original form of the test (devised in 1953) in which only the phospholipid concentration of the test was controlled(as opposed to the phospholipid and the surface activator concentrations) and the name partial thromboplastin was applied at the time to phospholipid preparation which accelerated clotting but did not correct the prolonged clotting times of haemophilic plasma. Essentially the term partial means phospholipid is present but no tissue factor.

The APTT also known as

1-Kaolin cephalin clotting time(KCCT)

2-Partial thromboplastin time with kaolin(PTTK)

Principles:Platlet poor plasma (PPP) is incubated at 37ċ then phospholipid (cephalin) and a contact activator e.g (Kaolin micronized silica or ellagic acid) are added followed by calcium (all pre-warmed to 37ċ) Addition of calcium initiates clotting and timing begins . the APTT is the time taken from the addition of calcium to the formation of a fibrin clot.

Reference ranges

Clotting time for the APTT lies between 27-35 sec.

Biochemical basis

Factor XII is produced which cleaves factor XI to XIa but doesn't proceed beyond this is in the absence of calcium. after recalcification factor XIa activates factor IX to IXa then IXa activates factor X to Xa in the presence of VIIIa , calcium and phospholipid ,then factor Xa generated cleaves prothrombin in a reaction catalyzed by factor Va ,phosoholipid and calcium ions ,liberating thrombin.

2.2.2.3.Tests of fibrinolysis:

Testing for hyperfibrinolysis by traditional tests, such as the euglobulin clot lysis times, is rarely performed. A clinically significant hyperfibrinolytic state, e.g. during liver transplantation, can be detected by viscoelastic measurement of clot stability using thromboelastography (TEG) or thromboelastometry (ROTEM) .

D-dimer is a measurement of fibrin degradation products and is an indication of sequential thrombin and then plasmin activity.

The test can be performed on citrated plasma samples along with simple coagulation tests. There are many causes of a high D-dimer, including infection, cancer and pregnancy, as well as venous thromboembolism. Plasma levels are very high in patients with disseminated intravascular coagulation (DIC).

2.2.2.4. Tests of Coagulation inhibitors

Include

1- antithrombin,

2-protein C .

3-protein S.

2-3 Previous Study

Several studies were done on women taking oral contraceptive pills :

1. One study was don on women taking oral contraceptive in Khartoum State-Sudan,byMohieldin Elsayid1, Mudathir Abdelrahim Mohammed Elbasheer2, Mahmoud Mohamed Elgari3, Tayseer Elamin Mohamed Elfaki4 . the results were showed significant shortened in TT and increased in APTT when compared with control group with P value < 0.05, and no significant variations were noticed in both PT and fibrinogen level with P value > 0.05 and this indicate the hypercoagulability. No significant changes were noticed between age groups, type and duration of oral contraceptives.
2. Other study was don in Bombay hospital to compare between changes in coagulation mechanism and duration of usage of pills, this study involved three groups of women –group A who were women doesn't use oral contraceptive as controls, group B user of combined oral contraceptive for 1,5-5 years, and group C who were women using pills for more than 5 years , significant changes occurred in APTT- Platelet count- - Platelet aggregation and PT, group B had higher PT and APTT than group A. They also showed arise in platelet aggregation ,platelet count were higher in both groups of user oral contraceptive than in control.
3. Other study in Tertiary Health Facility in Sokoto, North Western, Nigeria byErhabor O1*, Isaac IZ1, Kaoje AU2, John RT3 and Suleiman SA1,the result: a total of43 clients on hormonal contraceptives constituted the subjects. Twenty non-hormonal contraceptives users weremonitored as controls. PTTK and PT was carried out on citrated samples from subjects and control participants the results did not demonstrate any significant difference between the coagulation parameters of contraceptive users and control group ($t = 0.702$, $p = 0.491$ for

PT; t = 1.732,p = 0.100 for PTTK). There was significant differences in the in the PT and PTTK values of hormonal contraceptiveusers based on age. There was a negative correlation between PT and duration of contraceptive use.

4. A study was done by Rashida and Khalid in maternity clinic of the general hospital in Kualalumpur to study the effect of low dose of combined pills on coagulation, pills users showed shortening of PT and PTT but there were no significant change in factor II-V-VII-VIII.
5. Another one was studied the change in coagulation factors among pills users by NORRISL L A and BONNER J and it showed there is linked between oral contraceptive and increased incidence of thrombo vascular disease.

   This may be mediated by their effects on the haemostatic system. a significant increase in the coagulation factors VII-X and fibrinogen occur with combined pills usage. Increased factor VII level are dependent on both estrogen and progestogen component of oral contracepting.

   A reduction in antithrombin III levels has also been shown in oral contraceptive users which should balance the changes in the coagulation pathway.
6. Other study to Assessment of Coagulation Disorder in Women Taking Oral Contraceptives don by

Samsunnahar1, Qazi Shamima Akhter2, Atiquzzaman3, Najneen Akhter4,Umme Sultana Naima Begum5, Farhana Rahman6

The result is: The mean (±SE) totalcount of platelet level was significantly higher ($P<0.001$) in contraceptive user group and plasma fibrinogen level was higher in users but it was not significant.

Chapter Three

Methodology and Methods

3.1. Study design

A cross-sectional descriptive study conducted in Shendi locality during the period from Apr 2018 to July 2018 to evaluate the effects of oral contraceptive pills on the some coagulation tests (Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and platelet count.

3.2. Study area and sample

The study was conducted in Shendi town in Sudan, during the period between April to August2018 . Shendi is a town in northern Sudan, situated on the east bank of the Nile 150 km northeast of Khartoum. Shendi is also about 45 km southwest of the ancient city of Meroe. Located in the River Nile state, Shendi is the center of the Ja'aliin tribe and an important historic trading center. Its principal suburb on the west bank is Al-Matamma. A major traditional trade route across the Bayuda desert connects Al-Matamma to 1Marawi and Napata, 250 km to the north west. Sample size of 80 venous blood collected from oral contraceptive users and 40 from non users.

3.3. Study population

Eighty females from Shendi locality who under oral contraceptive pills, and forty as a controls.

3.4. Inclusion criteria

All females who were under oral contraceptive pills and resident in Shendi locality were enrolled.

3.5. Exclusion criteria

Presence of other coagulation disorders, pregnancy, and unused of oral contraceptive pills will exclude.

3.6. Ethical consideration

The consent of the selected individuals to the study was taken after being informed with all detailed objectives of the study and its health benefit in future.

3.7. Data collection

Data were collected using self-administered pre-coded questionnaire which was specifically designed to obtain information.

3.8. Data analysis

The analysis of the data was don using statistical package for social sciences (SPSS)computer programme.

3.9. Data presentation

The data were presented in tables

3.10. Sample

. The non probability sampling method was used in collecting samples .from each women 5 ml of venous blood was obtained to be divided in tow containers-one containing T.S.C(2g|l)in ratio1:9 0 was used and other containing EDTA, platelet poor plasma was obtained by centrifuging the TSC blood , for 15 minutes at 3000 rpm to estimate PT and APTT by coagulometer ,platelet was counted from anti coagulated blood by EDTA by hematology analyzer .

3.11. Matrial and equipment

- Disposable plastic syringe (.
- Cotton.
- 70%alcohol.
- 32%T.S.C-anticoagulant.
- EDTA- anticoagulant.
- Plan containers.

- 75×10mm glass tubes.
- 100-300ml automated pipette.
- Centrifuge.
- Hematology analyzer.
- Coagulometer.

3.11.1. Hematology analyzer:Mindray bc-3000 Principle

The analyzer measures the number of cells and distinguishing between their types according to size using sheath flow DC detection. Electrical current is passed through solution, this methods measured the change in electrical resistance that occurs when blood cell pass through detection aperture. this instrument performs hematology analyses according to the RF/DC detiction method.

3.11.2. The coagulometer

The automatic coagulometer (Clot) is an instrument for the determination of the main parameters used in the plasma coagulation methods.

Theory and principle:

The coagulometer (Clot) has an optical measurement system which detects a sudden variation in optical density when a clot is formed. The chronometer and the stirring system are activated by a sudden change of the optical density. This permits the initiation of the time measurement when the sample is added to the reagent and stop the measurement time at the moment that the clot is formed. The continuous mixing guarantees a perfect homogenization and makes the measurement possible of low concentrations of fibrinogen by grouping the fibrin filaments in the centre of the optical pass. The system has a programmable security time during which variations in optical density, when the reagent and the plasma are still in the homogenization phase, cannot activate the detection cell.

Procedure

First of all, the cuvette was placed corresponding to the determinations that were done on the thermostat. A magnetic stirrer was inserted in every cuvette and waited for the instrument to reach 37oC. After that, into the cuvette the reagent or sample volumes required were introduced. PT = (200 µl of reagent). APTT= (100 µl of reagent and 100 µl of plasma). When the thermostatisation time is finished, the cuvette was placed on the reading well. The chronometer was remained inactive for some seconds and then it was showed 000:0. At this moment the reagent or plasma was added with a disposable tip pipette, the liquid was left to get down with one blow and all the reaction was started at the same time. 100 µl of the starter was added: PT-Plasma APTT-Calcium Chloride . When the reagent and the plasma were in contact an O.D. variation was produced, that automatically activated the digital chronometer and the magnetic mixer. When the clot was starts to formed, an O.D. variation was produced and stopped the chronometer and the mixer. The clotting time appeared on the display.

3.12. Methodology of test performed

3.12.1.Prothrombin Time:

Principle

The PT test measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. Although originally thought to measure prothrombin, the test is now known to depend also on reactions with factors V, VII, and X and on the fibrinogen concentration of the plasma .

Reagents

A)Tissue thromboplastin: Thromboplastins were originally tissue extracts obtained from different species and different organs containing tissue factor and

phospholipid. Because of the potential hazard of viral and other infections from handling human brain, it should no longer be used as a source of thromboplastin. The majority of animal thromboplastins now in use are extracts of rabbit brain or lung.

B)Buffer with calcium ions and sodium azide as preservative.

Reagent preparation

reagent B is ready to use.

Working Reagent: pour the content of reagent B into the reagent A.mix gently and keep at 18-25c for 30 minutes.

Procedure

1) __Bring the working reagent to 37c.
2) Pipette 50ul of of sample to the test tube.
3) Incubate sample at 37c for 2 minutes.
4) Pipette 100ul of working reagent and simultaneouslystartstopwatch.
5) Determine the coagulation time.

3.12.2 .Activated Partial Thromboplastin Time

Other forms of the APTT test are known as the partial thromboplastin time with kaolin (PTTK) and the kaolin cephalin clotting time (KCCT), reflecting the methods used to perform the test.

Principle

The test measures the clotting time of plasma after the activation of contact factors but without added tissue thromboplastin and so indicates the overall efficiency of the intrinsic pathway. The test depends not only on the contact factors and on factors VIII and IX, but also on the reactions with factors X, V, prothrombin, and fibrinogen. It is also sensitive to the presence of circulating anticoagulants (inhibitors) and heparin

Reagents:

1) Kaolin: 5 g/l (laboratory grade) in barbitone buffered saline, pH 7.4.A few glass beads were added to aid resuspension. The suspension is stable at room temperature. Other insoluble surface active substances such as silica, celite, or ellagic acid can also be used.
2) Phospholipids:Cephalin as phospholipids substitution.
3) CaCl2: 0.025 mol/l.

Preparation And Stability

- Use freshly Collected blood taken into 0.11mol|l trisodium citrate in the ratio 9 parts blood to 1 part anticoagulant.
- Centrifuge immediately for 5 minutes at RCF 1500-2000 g and separate plasma into a clean test tube.
- Plasma should be tested within 3 hours (keep refrigerated).

PROCEDURE

1. Pre-incubate the calcium chloride reagent to 37ct for at least10 minutes .pipette 100ul of test or control plasma into attest cuvette.
2. Incubate the plasma at 37c for 1 to 2 minutes.
3. Pipette 100ul of the APTT reagent by magnetic stirring or mixing by inversion immediately prior to use.
4. Incubate at 37c for 3 minutes.
5. Add 100ul preinubated calcium chloride solution and simultaneously start the timer.
6. Record the clotting time in seconds.

3.12.3 Platelet Count

Automated count. Platelets can be counted in whole blood anticoagulated by EDTA.

Chapter Four

Results

4.1. Results

When compared the mean of PT, APTT, and platelet count results between study group and control group, insignificant difference in mean PT result between cases (14.3 seconds) and control (13.2 seconds) (P value= 0.08), significant increase of mean APTT result (32.1 seconds) when compared with mean APTT of control (29.7 seconds) (P value = 0.003), significantly higher in mean of platelet count result in cases (519.) when compared with control (272.) (P value = 0.004) in table one.

When measured the effect of age group on (PT, APTT, and platelet count) results, there were insignificant difference in mean PT result in study group of age less than 30 years (13.6 seconds) and of age more than or equal 30 years (14. seconds) (P value =0.35), significant difference in mean APTT result in study group of age less than 30 years (33.5 seconds) and of age more than 30 years (36.2 seconds) (P value =0.05), there were significant difference in mean.

Platelet count result in study group of age less than 30 years (290)and of age more than or equal 30 years(535)(P value =0.04)in table tow.

The effect of contraceptives duration on PT, APTT, and platelet count results was measured, there were insignificant difference in mean PT result between contraceptive duration of(5months-2years) (13.8 seconds +) and of (2yers-5yers) (14.0 seconds) (P value = 0.92), significant difference in mean APTT result between contraceptive duration of 5months-2years (30.5seconds) and of2yers-5yers (35.4seconds) (P value = 0.007) ,in significant difference in mean platelet count result between contraceptive duration of 5month to2years(316)and of2yers-to5yers(322)(Pvalue=0.65) in table three.

The effect of contraceptives type on mean (PT, APTT, platelet count):

results was measured, there were significant difference in mean PT result between usage of combined oral contraceptive (COC) (15seconds) and usage of progestin only pills (POP)mini pills-(13.2 seconds) (P value = 0.03), significant difference in mean APTT result between usages of combined oral contraceptive (COC) (36.2 seconds) and usage of progestin only pills (POP) (34.5 seconds) (P value = 0.01), significant difference in mean of platelet count result between usages of combined oral contraceptive(505) and usages of mini pills oral contraceptive (290+SD), (P value =0.007).

Table (4-1)
Mean of PT,PTT and platelet count in women use contraceptive and control

Parameter	Sample	Mean	P. value
PT/ **seconds**	Case	14.3	0.08
	Control	13.2	
APTT| seconds	Case	32.1	0.003
	Control	29.7	
Platelet count	Case	519	0.004
	Control	272	

Table (4: 2)
Mean of PT, APTT, and platelet count results according to age

Parameter	Age group / year	Mean	P. value
PT / seconds	≤30	14.	0.35
	>30	13.6	
APTT/ seconds	≤30	36.2	0.05
	>30	33.5	
Platelet count	≤30	535	0.04
	>30	291	

Table (4 :3)
Mean PT, APTT, and platelet count results according to duration uses of contraceptive

Parameter	Duration group/years	Mean	P. value
PT / seconds	5m-2y	13.8	0.92
	2y-5y	14.0	
APTT/ seconds	5m-2y	30.5	0.007
	2y-5y	35.4	
Platele count	5m-2y	316	0.65
	2y-5y	322	

Table(4: 4)
Mean of PT, APTT, and platelet count results in different types of oral contraceptive pills

Parameter	Type of Contraceptives	Mean	P. value
PT / seconds	Combination	15.0	0.03
	Mini pills	14.1	
APTT/ seconds	Combination	36.2	0.01
	Mini pills	34.4	
Platelet count	Combination	505	0.007
	Mini pills	290	

Chapter Five

- Discussion
- Conclusion
- Recommendati

5.1. Discussion

Prothrombin Time(PT)- Activated Partial Thromboplastin Time(PTT) and platelet count are useful parameter for the clinical assessment of patients with hypercoagulable state such as the women on oral contraceptive pills.

This is a descriptive cross-sectional study which was conducted in Shendi locality during the period of Apr 2018 to July 2018 to evaluate the effects of oral contraceptive pills (combined & progestin only pills) on the some hemostasis tests (Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and platelet count.

. A eighty sample were collected from females according to inclusion criteria and considered as case, and 40 samples were collected from females do not taken these pills, and considered as control. The results revealed that:

- The mean of PT result in study group was (14.3 seconds) and when compared with control(13.2 seconds) ,the result was insignificance (P value >0.05).

The mean APTT result of study group was (32.1 seconds) and when compared with control group (29.7 seconds) the result was significant with (P value<0.05).and the result was similar to study done in Khartoum State- Sudan by Mohieldin Elsayid and etal in2011which results of cases revealed that PT mean= 14.0 seconds , and APTT mean= 33.4 seconds .

In this study the mean of platelet counts in women of OCP users(519×10^9|l) were higher than that of non users(272×10^9|l) and the result was statistically highly significant(P valu<.05) and this agreement with study don by Samsunnahar1, Qazi Shamima Akhter2 etal ,its result The mean of total count of platelet was significantly higher (P<0.001) in contraceptive user .

- ❖ The mean of PT in study group who in age group of ≥ 30 years was (14 seconds)and when compared with age group of <30 years (13.6 seconds) :

The result was statistical insignificance (P value >0.05). his results was agreement with the study in khartoum state by Mohieldin Elsayid and etal- in 2011(there were insignificant difference in mean PT result in study group of age less than 30 years (14.1 seconds) and of age more than 30 years (13.8 seconds) (P value = 0.35).

The mean APTT in study group who in age group of ≥ 30 years was (36.2 seconds) and when compared with age group of <30 years (33.5 seconds) :the result was statistical significance (P value <0.05).

This results was diss agree with the study in Khartoum state by Mohieldin Elsayid and ctal- in 2011(there was insignificant difference in mean APTT result in study group of age less than 30 years (33.7 seconds) and of age more than 30 years (33.2 seconds) (P value = 0.52).

- mean Platelet count result in study group of age less than 30 years (290×10^9 |l) compared with group of age more than 30 years(535×10^9|l).the result was significant (P value <0,05).

- ❖ In this study the mean of PT in contraceptive duration of(5months-1years)= (13.8 seconds) and of (2yers-5yers) (14.0 seconds) the result was insignificant (P value >0.05),the mean APTT result in contraceptive duration of 5months-2years (30.5 seconds)when compared with that who used the pills for > 2yers (35.4 seconds ± SD) the result was in significance (P value >0.05).the mean of platelet count result between contraceptive duration of 5month to2years(316×10^9)and of2yers- to5yers (322×10^9).the result was insignificant (P value>0.05).

This study was disagree with previous study don in Bombay hospital to compare between changes in coagulation mechanism and duration of usage of pills, it's

result: significant changes occurred in APTT- Platelet count- - Platelet aggregation and PT.

- In this study the men of PT result between usage of combined oral contraceptive (COC) (15seconds) and when compared with usage of progestin only pills (POP)mini pills-(13.2 seconds) the result was significant (P value <0.05).the mean APTT result between usages of combined oral contraceptive (COC) (36.2 seconds) and when compared with usage of progestin only pills (POP) (34.5 seconds);

the result was significant (P value <0,05).the mean of platelet count result between usages of combined oral contraceptive(505×10^9) and usages of mini pills oral contraceptive (290×10^9) .the result was significant (P value < 0.05).

- This study was correlate with study don by Rashida and Khalid in maternity clinic of the general hospital in Kualalumpur to study the effect of low dose of combined pills on coagulation, pills users showed shortening of PT and PTT but there were no significant change in factor II-V-VII-VIII.

5.2. Conclusion

The result obtained from this study concluded that:

a) The mean of PT is14.3|s.
b) The mean APTT is 32.1|s.
c) The mean of platelet count is 519.
d) There was effect of age and type ofcontraceptive on coagulation profile.
e) This study concludes that OCP users had more tendency of hypercoagulability and therefore these women are at higher risk of thromboembolic

5.3. Recommendation

- Further studies to validate our findings should be carried out on larger number of subjects using probability sampling techniques.
- Further tests like protein C – protein S –antithrombin -fibrinogen and fibrin D-dimer must be measured in these group of pills users to confirm ahypercoagulable state.
- Follow up women who use the combined oral contraceptive pills for along period of time to detect the relationship between the changes in the coagulation factors and duration of the use and detect any thromboembolic phenomena that may occur in them.
- Doctors should be alerted to the possible effects of combined pills despite the fact that they are more effective in contraceptive.
- Use Other contraceptive that safe rather than oral contraceptive.

Chapter Six

References

Appendices

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بسم الله الرحمن الرحيم

Shendi University

Faculty of Medical Laboratory Science

Program of Master Hematology

Determination of Oral Contraceptive pills Effect on Coagulation Profile among Sudanese women in shendi locality

Age……..

Obese: yes(……)……-

NO(……)……

Hyper tenser: yes(……)……NO(……)……

Diabetes: yes(……)……NO(……)……

Previous history of thrombosis:

-yes(……)……NO(……)……..

Previous history of bleeding…-

yes(……)……NO(……)……

Heart failure:…yes(……)……NO(……)……

Migraine:yes(……)……NO(……)……

Duration of used: 5m( )-…… 1year( )……2year( )

3year( )……..other( )

Appendix1

Composition :

- EDTA

Ethylene diamin tetra acetic acid

Dipotassium salt 100g

Water 1litter

- Trisodium citrate($Na_3C_6H_5C_72H_2O$)
  109 mm| ml :32 g in one litter of water

Appendix 2

$$T = \frac{X - X /}{\frac{S}{N} + \frac{S}{N}}$$

T: Calculated values which are found out from our ostentation.

X_1: Mean of the first group.

X_2: Mean of the second group.

S_1^2: Variance of the first group.

S^2_2: Variance of the second group.

N_1:Numper of the sample in the first group.

N_2:Numper of the sample in the second group.

Tabulated value=1.96

If the calculated value is moor than tabulated value the difference insignificant and vice versa.