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The Effect of Oral Contraceptive Pills on Platelets Count and D-dimer Level in Shendi Town

A thesis submitted in The partial fulfillment For the Requirements of the degree of Master in medical Laboratory Sciences (Hematology)

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الآية

بسم الله الرحمن الرحيم

قال الله تعالي:

﴿ اقرأ وربك الأكرم ۝٣ الذي علم بالقلم ۝٤ علم الإنسان ما لم يعلم ۝٥ ﴾

سـورة العلـق

DEDICATION

I dedicate this thesis

To the spirit of my father, God has entered into his paradise.

To my great mother, who continue to learn, grow and develop and who have been a source of encouragement and inspiration to me throughout my life.

To my dear husband who gave me love and support.

To my self, who challenged all disincentives to get the master degree.

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List of Abbreviations

Abbreviation	Term
ADP	Adenosine Diphosphate
AGM	Aorta-gonads-mesonephros
ATP	Adenosine Triphosphate
BSA	Bovine Serum Albumin
CBC	Complete Blood Count
COC	Combined Oral Contraceptive
DC	Direct Current
COCPs	Combined Oral Contraceptive pills
DNA	Deoxyribonucleic acid
DSG	Desogesterol
DTS	Dense Tubular System
EDTA	Etyline Diamine Tetcra acetic Acid
EE	Ethinyl Estradiol
EM	Electrone Microscope
FSH	Follicle – stimulating hormone
GP	glycoprotein
IgA	Immunoglobin A
IgG	Immuno globin G
IgM	Immunoglobin M
LH	Lutenizing hormone
LNG	Levonorgesterol
MKs	Megakaryocyte
OCS	Open Canalicular Systenm
OCs	Oral contraceptive
PAR	Protese activated receptor
PBS	Phosphate buffered saline
PDGF	Platelet-derived growth factor
PGG2	prostaglandin G2
PGH2	prostaglandin H2
PL	phospholipid
POPs	Progestin –Only Pills
RF	Radio Frequencies

SLS	Sodium Lauryl Sulphate
SPSS	Statistical Package for Social Sciences
TPO	Thrombopoietin
TXA2	Throboxane A2
VTE	Thromboembolism
VWF	Von Willebrand Factor

Abstract

Background: Oral contraceptive pill (OCP) is related to development of hypercoagulability and the risk of thromboembolic effects in women.

Aim of the study: This study aimed to assess the effect of oral contraceptive on platelet count and D-dimer level in women in Shendi Town.

Methods: A cross sectional study was conducted during the period from April to December 2018. To detect the effect of oral contraceptives on platelets count and D-dimer level among 50 women using oral contraceptives pills (as case group) and 30 women not using contraceptive as a control group. The platelet count were estimated by automated hematology analyzer and D-dimer level estimated by Ichroma™. Statistical analysis was done by SPSS.

Results: The platelet count was significantly increased in the oral contraceptive users group compared to the control group (P .Value=.028). There were also strong significant statistical value increase in D-dimer levels in the oral contraceptive users group compared to the control (P.Value=0.000).

Cconclusion : This study concludes that OCP users had more tendency of hypercoagulability due to increase in platelets count and high D-dimer level and there fore these women are at higher risk of thromboembolic effects.

الملخـــص

المقدمة: موانع الحمل عن طريق الفم هي ذات الصلة لتطوير فرط التخثر وخطر الانسداد التجلطي عند النساء.

الهدف: الهدف من هذه الدراسة هو قياس تأثير موانع الحمل الفموية علي عدد الصفائح الدموية ومستوى الدى ديمر عند النساء في مدينة شندي.

الطريقة: هذه دراسة تحليلية وصفية مستعرضة أجريت خلال الفترة من شهر ابريل إلى شهر ديسمبر في سنة 2018م. وقد كانت اكتشاف تأثير موانع الحمل الفموية على عدد الصفائح الدموية ومستوى دي ديمر في 80 امرأة كانت منهن 50 امرأة تستخدم موانع الحمل الفموية و30 امرأة لا تستخدم موانع الحمل الفموية كمجموعة ضابطة.

لعد الصفائح الدموية تم استخدام جهاز محلل الدم الكامل، وقد تم قياس مستوي دى ديمر بجهاز آى كروما.

أما التحليل الإحصائي فقد تم بواسطة برنامج (الحزمة الإحصائية للعلوم الاجتماعية).

النتائج: أظهرت الدراسة أن هناك زيادة ذات دلالة إحصائية في عدد الصفائح الدموية عند النساء المستعملات لحبوب منع الحمل مقارنة مع المجموعة الضابطة بقيمة معنوية هي (0.028)، كما وجد أن اختبار دي ديمر كان مرتفعا عند نفس النساء بقيمة معنوية (0.000). **الخلاصة:** خلصت هذه الدراسة إلي أن مستخدمي موانع الحمل الفموية لديهم ميل أكبر للتخثر، وبالتالي فإن هؤلاء النساء أكثر عرضة لخطر الانسداد التجلطي والتي ترتبط بزيادة ذات دلالة إحصائية في عدد الصفائح الدموية ومستوي دي ديمر.

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1.1 Introduction

The rapid increase in human population is causing great concern on the challenge of sustaining associated increased demand for resources such as fresh water, food natural resources and living conditions. Contraception has been identified as one of the ways to mitigate adverse effects associated with the overpopulation [(1)]

Hormonal contraception is a birth-control method to prevent ovulation and thus pregnancy. Hormonal contraception consists of steroid hormone use in two main types of formulations; combined formulations which contain both estrogen and progestagen and progestagen-only formulations. Progestagen suppresses the surge in luteinizing hormone (LH) and thereby prevents ovulation. Estrogen reduces the secretion of follicle-stimulating hormone (FSH) and thereby inhibits folliculogenesis. The estrogen compound has a major role in drug compliance; by increasing the stability of the endometrium, breakthrough bleeding and spotting are reduced. Hormonal contraception is prescribed to regulate the uterine, menstrual cycle or for other hormonally dependent disorders as acne and hairsutism . [(2)]

Oral contraceptive pills have undesired side effects such as gastric upsets, changes in body weight and allergic skin reactions have been reported .More seriously their use is known to be associated with a 3-6 fold greater risk of developing thromboembolic disease especially when associated with other risk factors like obesity and age.This high risk is attributed to their high oestrogen content which causes a hypercoagulable state by leading to a rise in the level of coagulation factors such as11, VII, VIII,VwF, IX, X, XI and fibrinogen [(3)].

The use of oral contraceptives (OCs) has been associated with vascular complications. [(4)] 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 factors, platelets changes and thromboembolic phenomenon [5]

Platelets are small anucleate cell fragments that circulate in blood playing crucial role in managing vascular integrity and regulating hemostasis. Primarily, platelets activity is associated with the initiation of coagulation cascades. Damage in blood vessel makes the subendothelial surface the primary target site of platelets action, where it establishes the hemostasis. [6]

In the early 1980s the first monoclonal antibody–based assays for D-dimer, a specific fibrin(ogen) degradation product, were described that provided an assay with greater specificity for fibrin proteolysis. The clinical conditions associated with elevated levels of D-dimer are numerous. Some of these include thrombosis (arterial or venous), pulmonary embolism, venous thrombosis, disseminated intravascular coagulation, myocardial infarction, stroke, postoperative state,liver disease, malignancy, and pregnancy. [7]

1.2 Rationale

Oral contraceptive use is known to cause change in haemostatic system. These changes are thought to be related to estrogen dose and to provide apossible link of thromboembolic disease known to occure in women taking oestrogen containing oral contraceptive.

The platelets play an important role in blood clotting and haemostasis. Increase or decrease of platelets count affects normal haemostasis [18].

There are many studies reported that contraceptives have effect on platelets count and cause some coagulation disorders.and it is association with the risk of both venous thrombosis and arterial disease .

Therefore this study was conducted to assess the effect of oral contraceptive pills on platelets count and D-dimer level in women using oral contraceptive in Shendi Town.

1.3 Objectives

1.3.1 General objective

To detect the effect of oral contraceptive on platelets count and D-dimer level.

1.3.2 Specific objectives

1. To compare platelets count and D-dimer level in women using contraceptives and women not using oral contraceptives.
2. To compare the age and duration of oral contraceptives with platelets count.
3. To compare the age and duration of oral contraceptives with D-dimer level.
4. To compare between platelets count and D-dimer level according breast feeding.
5. To determine the effect of number of pregnancies on platelets count and D-dimer in women use oral contraceptive pills.

Chapter Tow

Literature review

2. Literature Review

2.1 Contraceptives

Contraceptive, family planning or birth control prevents pregnancy by barrier methods that physically prevent sperm and egg from meeting, hormonal methods that prevent ovulation, and behavioral such as abstinence around the time of ovulation. It used to limit family size and space births [8]

2.1.1 Hormonal contraception

Can include a combination of estrogen and progestin, or progestin alone. It can be administered orally, injection, intravaginally, implant, or intrauterine device. Progestin inhibit ovulation by suppressing luteinizing hormone (LH), thickening of cervical mucus hampering the transport of sperm. Oral contraceptives block ovarian stimulation by prevent release of follicular stimulation hormone from anterior pituitary gland and prevent ovulation [9]

2.1.1.1 Progestin

The progestin causes the cervical mucus to thicken and become viscid and scant. These actions inhibit sperm penetration into the uterus. Also progestin impairs the motility of the uterus and oviducts and therefore decrease transport of both ova and sperm to the normal site of fertilization in the distal fallopian tube. Progestin also produce changes in the endometrium that are not conducive for implantation of the embryo.[10]

2.1.1.2 Estrogen

Estrogen increase the thickness of the endometrium by increasing the number and size of the endometrial cells. It also stimulates the formation of progesterone receptors on endometrial cells and increase blood flow to the endometrium. Estrogen is change the cervix and cervical mucus and prevents the sperm from entering the uterine. [11]

2.1.1.3 oral contraceptive advantages

Modern oral contraceptives afford not only excellent contraception but also a variety of non-contraceptive benefits, ranging from regulation and reduction of both menstrual bleeding and dysmenorrhea to treatment of premenstrual syndrome, menstrual migraines, acne, and hirsutism. Long-term benefits include reduced rates of endometrial, ovarian, and colorectal cancer.[12]

2.1.1.4 oral contraceptive disadvantage

menstrual spotting or missed periods, nausea, mild headaches, breast tenderness, slight weight gain or loss, and mood changes. [13]

2.2 Type Of Oral Contraceptive

- Combined oral contraceptive[COC] and
- progestin-only pills (POPs).

2.2.1Combined oral contraceptives "the combined formulation of estrogen and progestins are widely used as a temporary contraceptives; however their safe use has been remained under debate. Combined oral contraceptives containing less than 50 μg ethinyl estradiol has been named as low dose oral contraceptives [5]. The first marketed COC consisted of high doses of synthetic estrogen and androgenic progestin such as norethisterone acetate or norethindrone. The current COCs now deliver 15– 50 mg of EE/day and new formulations delivering natural EE have recently been marketed. A quadriphasic COC combining EE valerate and dienogest has been newly approved in Europe and USA and a second monophasic COCs that combines EE with nomegestrol acetate, aprogesterone-derived progestin, is now available in several countries in Europe.

COCs are also classified into generation, according to the type of progestin associated with estrogen [14]

2.2.2 Progestin-only pills "POPs or mini pills containing only progesterone.[3] deliver low daily doses of progestin (norethindrone, LNG, or

DSG). Although the original development of oral contraceptives focused on progestin-only products, current POPs are less used than COC because of their poorer uterine tolerance. The menstrual cycle is less well controlled, and bleeding such as spotting is common .Nevertheless, POPs may be an attractive contraceptive option for women with contraindications to COCs.[14]

2.3 Oral contraceptive and risk of thrombosis

A causal relationship between the use of oral contraceptives and thromboembolic disease was first described in the united kingdom in 1967 and in clinical trials of Grant 1969 found that leg vein complaints including thrombophelbitis occurred most frequently with combined preparation containing low dose of progestogen and high dose of estrogen .

Estrogen therapy especially with high doses of synthetic estrogen in OCs has been shown to increase platelets aggregation and to enhance clot formation . The dose of reduction of estrogen component in OCs has results in a concomitant decrease in the incidence of thromboembolic disease [4]

Combined oral contraceptives (COCs) are a common method of contraception, but they carry a risk of venous and arterial thrombosis. The association between estrogen-containing oral contraceptives (OCs) and venous thromboembolism (VTE) is well established [15]. Until 1995, it was generally thought that the progestogen component of COCs did not contribute to the risk of VTE. However, a number of studies published in late 1995 and early 1996 reported an increased risk of VTE for users of the so-called third-generation COCs containing the progestogens, desogestrel or gestodene, compared with second-generation COCs, containing levonorgestrel [16]. These findings were unexpected and led to debate over possible bias and confounding. New studies, re-analyses of the original studies and a meta-analysis have since been published [17].

2.4 Blood

Blood is composed of a pale yellow fluid called plasma in which are suspended red cells (erythrocytes), white cells (leukocytes), and platelets (thrombocytes). Plasma forms about 55% of blood volume and contains water (95%) and many solutes, including proteins, mineral ions, organic molecules, hormones, enzymes, products of digestion, and waste products for excretion. Blood flows through every organ of the body, providing effective communication between tissues. It is kept in continuous circulation by the pumping action of the heart, flowing through arteries which carry the oxygenated (bright red) blood from the heart to all parts of the body, and veins which carry the deoxygenated (dark red) blood from the different parts of the body back to the heart and to the lungs. The arteries divide into smaller vessels called capillaries forming the capillary, or peripheral, circulation which supplies oxygen to the tissues. The capillaries rejoin to form the veins.[18]

2.4.1 Function of blood

The Main functions of the blood Blood has important transport, distribution, regulatory, and protective functions in the body. [18]

2.4.2 Blood cell production

Formation of blood cells occurs at different anatomical sites during the course of development from embryonic to adult life [19]. In the first few weeks of gestation the yolk sac is a transient site of haemopoiesis. However, definitive haemopoiesis derives from a population of stem cells first observed on the AGM (aorta-gonads-mesonephros) region. These common precursors of endothelial and haemopoietic cells (haemangioblasts) are believed to seed the liver, spleen and bone marrow. From 6 weeks until 6–7 months of fetal life, the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth. The placenta also contributes to fetal haemopoiesis. The bone marrow is the most important site

from 6–7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses; mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation[20]

2.4.3 Control of blood cell production

Haematopoiesis is regulated by a range of cytokines (growth factors) which include interleukins, stem cell factors, colony stimulating factors, erythropoietin and thrombopoietin. They interact with specific receptors on the surface of haematopoietic cells, regulating the proliferation and differentiation of progenitor cells, and the maturation and functioning of mature cells. [18]

2.4.4 platelets

Blood platelets are small, anucleate cellular fragments that play an essential role in hemostasis. During normal circulation, platelets circulate in a resting state as small discs. However, when challenged by vascular injury, platelets are rapidly activated and aggregate with each other to forma plug on the vessel wall that prevents vascular leakage. Each day, 100 billion platelets must be produced from megakaryocytes (MKs) to maintain the normal platelets count of 2 to 3 × 10/mL [21]

2.4.4.1Platelets 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 [20]

2.4.4.2 Regulator Of platelets Production

The major regulator of platelets production is the hormone thrombopoietin (TPO) [22], 95% is produced by the liver [20]. TPO binds to its receptor on platelets and megakaryocytes, by which it is removed from the circulation. Thus, a reduction in platelets and megakaryocyte mass increases the level of TPO, which then stimulates platelets production. **[22]**

2.4.4.3 platelets circulation

Platelets circulate with an average life span of 7 to 10 days. Approximately one-third of the platelets reside in the spleen, and this number increases in proportion to splenic size, although the platelets count rarely decreases to <40,000/μL as the spleen enlarges. platelets are physiologically very active but are anucleate and thus have limited capacity to synthesize new proteins [22]

Splenomegaly, particularly when caused by passive congestion due to increased portal venous pressure, greatly increases the fraction of platelets retained in splenic sinusoids, without decreasing overall platelets urvival time. This retention causes the mild thrombocytopenia associated with liver cirrhosis and portal hypertension. Most platelets are removed from the circulation after senescence, but a constant small fraction is continually removed by involvement in the maintenance of vascular integrity. [23]

2.4.4.4 platelets tructure and morphology

platelets are small (2–3 mm), anucleate, disc-shaped cells of which two-thirds are present in the general circulation with the remaining third reversibly sequestered in the spleen. platelets contain a number of distinguishable structural elements including: a delimited plasma membrane; invaginations of the surface membrane that form the open canalicular system (OCS); a closed-channel network of residual endoplasmic reticulum that form the dense tubular system (DTS); a spectrin-based membrane skeleton; an actin-based cytoskeletal network; a peripheral band of microtubules; and numerous organelles including a-granules, dense-granules, peroxisomes, lysosomes, and mitochondria [(24)]

2.4.4.4.1Ultrastructure of platelets

Ultra structurally, following three zones can be distinguished:

2.4.4.4.1.1 Peripheral zone: contain:

- exterior coat (glycocalyx)
- cell membrane
- open canalicular system

Exterior or surface coat (glycocalyx) overlies the cell membrane. It is made of proteins, glycoproteins, and mucopolysaccharides. Some of the glycoproteins are polysaccharide side chains of the integral membrane proteins while others are adsorbed from the plasma. The cell membrane is a trilaminar membrane composed of proteins, lipids, and carbohydrates. The chief membrane lipids are phospholipids which are arranged as a bilayer; the polar head groups are oriented both externally (towards plasma) and internally (towards cytoplasm) while the fatty acid chains are oriented toward each other. Phospholipids are distributed asymmetrically in the membrane with phosphatidylinositol concentrated on the inner half of the bilayer and phosphatidylethanolamine on the outer half. The phospholipids play an important role in prostaglandin

synthesis and in platelets procoagulant activity [25]. The open canalicular system (OCS).

Is the "tunnel" system present throughout the platelets cell and remains connected with the plasma membrane [6]. The major role of OCS is to give entry of external elements into the platelets as well as to release its granule contents to the exterior. Other than being a major storage site for plasma membrane glycoproteins, it facilitates the formation of filopodia during platelets activation [6].

The cell membrane contains integral membrane glycoproteins (Gp), which play an important role in haemostasis. Important platelets membrane glycoproteins and their functions are as follows: *Gp Ib-IX-V:* This is a constitutively active receptor that mediates vWF-dependent adhesion of platelets to sub endothelial collagen. Gp IIb/IIIa*:* On activation, serves to bind fibrinogen and thus mediates aggregation. Also receptor for vWF, fibronectin, and thrombospondin. *Gp Ia-IIa:* Constitutively active receptor for collagen and mediates platelets adhesion independent of vWF. [25]

2.4.4.4.1.2 Sol-gel zone

- microfilaments
- circumferential microtubules
- dense tubular system

This is cytoplasm corresponding part of the cellular fragment, platelets . It is in soluble or gel phase according to changes of polymerization of the filaments, actin and microtubules [26] .Just under the sub membrane zone there are microtubules forming a peripheral ring which helps platelets to maintain its discoid shape in inactive form. When activated**,** the microtubules surround the organelles and with the contribution of other filaments [27] ,the organelles are tightly contracted. During silent form only 30-40 % of actin filaments are polymerized, when platelets are activated the polymerized amount increases[26]

Dense tubular system of platelets is a closed-channel network of residual endoplasmic reticulum and primarily involved in calcium sequestration with the help of cascades of reactions involving the activation of G protein-coupled receptor PAR-1 [6]

2.4.4.4.1.3 Organelle zone platelets organelles are:

- alpha granules
- dense granules
- lysosomes
- mitochondria and peroxisomes [25]

2.4.4.4.1.3.1 Alpha-Granules

Th e more frequent specifi c α granules contain clotting factors,VWF, platelets - derived growth factor (PDGF) and other proteins [28], alpha-Granules are present in the earliest recognizable cell of the megakaryocytic series, before the development of extensive demarcation membranes. alpha-Granules are thought to be derived from the megakaryocyte Golgi complex during cell maturation These spherical to oval granules measure 200-500 nm in diameter and contain a nucleoid within a finely granular matrix. They contain numerous protein constituents. Both integral membrane glycoproteins and granule content proteins have been identified. platelets formation includes partitioning of a-granules into megakaryocyte cytoplasmic processes that are believed to represent the first step in the platelets birth process. [29].

2.4.4.4.1.3.2 Dense Granules

Dense granules (or dense bodies), 250 nm in size, identified in electron micrographs by virtue of their electron-dense cores, function primarily to recruit additional platelets to sites of vascular injury. Dense granules contain a variety of hemostatically active substances that are released upon platelets activation, including serotonin, catecholamines, adenosine 5-diphosphate (ADP), adenosine 5-triphosphate (ATP), and calcium. Adenosine diphosphate is a strong platelets agonist, triggering changes in the shape of platelets , the

granule release reaction, and aggregation. Recent studies have shown that the transport of serotonin in dense granules is essential for the process of liver regeneration. Immunoelectron microscopy studies have also indicated that multivesicular bodies are anintermediary stage of dense granule maturation andconstituteasortingcompartmentbetweenα granules and dense granules. (30)

2.4.4.4.1.3.3 Mitochondria

platelets contain a small number of mitochondria that are identified in the electron microscope by their internal cisternae. They provide an energy source for the platelets as it circulates in the bloodstream for 7 days in humans.(21)

2.4.4.4.1.3.4 Peroxisomes are small organelles that contain the enzyme catalase (21)

2.4.4.4.1.3.5 Lysosomes They have a diameter of 200-250 nm which places them to middle size granule. They can't be distinguished from alpha granules under EM observation because of the similarities in dense electron appearance. By the content of acid phosphates and arylsulphates cytochemical staining techniques can effectively distinguish lysosomes from alpha granules. In an activated platelets they expel their contents to environment as the other two granules by membrane fusing mechanisms. The difference for lysosomes to be involved in activation is that they need a more potent stimulus. The role of lysosomal components in homeostasis is not well understood as the other granules contribution. They are involved in thrombus formation and extracellular matrix remodeling ,It seems that lysosomes in platelets don't have any distinguished features, they share the common features with other cells lysosomes (31)

2.4.4.5 Role of platelets in Haemostasis

Hemostasis is a dynamic process in which the platelets and the blood vessel wall play key roles. platelets become activated upon adhesion to von Willebrand factor (vWF) and collagen in the exposed sub endothelium after

injury. platelets activation is also mediated through shear forces imposed by blood flow itself, particularly in areas where the vessel wall is diseased, and is also affected by the inflammatory state of the endothelium. The activated platelets surface provides the major physiologic site for coagulation factor activation, which results in further platelets activation and fibrin formation. Genetic and acquired influences on the platelets and vessel wall, as well as on the coagulation and fibrinolytic systems, determine whether normal hemostasis or bleeding or clotting symptoms will result. [22]

2.4.4.6 platelets Function

2.4.4.6.1 Adhesion This means binding of platelets to non endothelial surfaces, particularly sub endothelium which is uncovered following vascular injury. von Willebrand factor (vWF) mediates adhesion of platelets to sub endothelium via GpIb on the surface of platelets Congenital absence of glycoprotein receptor GpIb (Bernard- Soulier syndrome) or of von Willebrand factor in plasma (von Willebrand's disease) causes defective platelets adhesion and bleeding disorder. platelets normally circulate as round to oval disc-like structures. With activation, platelets undergo shape change, i.e. they become more spherical and form pseudopodia. This shape change is due to reorganisation of microtubules and contraction of actomyosin of microfilaments. [25]

2.4.4.6.2 Release reaction (secretion) immediately after adhesion and shape change, process of release reaction or secretion begins. In this process, contents of platelets organelles are released to the exterior. ADP released from dense granules promotes platelets aggregation. platelets factor 4 released from alpha granules neutralises the anticoagulant activity of heparin while platelets -derived growth factor stimulates proliferation of vascular smooth muscle cells and skin fibroblasts and plays a role in wound healing.

Activated platelets also synthesis and secrete thromboxane A2 (TxA2) platelets agonists such as ADP, epinephrine, and low-dose thrombin bind to

their specific receptors on platelets surface, and activate phospholipase enzymes, which release arachidonic acid from membrane phospholipids. Arachidonic acid is converted to cyclic endoperoxides PGG2 and PGH2 by the enzyme cyclo-oxygenase. These are then converted to thromboxane A2 by thromboxane synthetase. Thromboxane A2 has a very short half-life and is degraded into thromboxane B2 which is biologically inactive. TxA2 causes shape change and stimulates release reaction from alpha and dense granules. TxA2 also induces aggregation of other platelets and local vasoconstriction.[25]

2.4.4.6.3 Aggregation This may be defined as binding of platelets to each other. ADP released from platelets or from damaged cells binds to specific receptors on platelets surface. This causes inhibition of adenyl cyclase and reduction in the level of cyclic AMP in platelets . A configurational change in the membrane occurs so that receptors for fibrinogen (GpIIb and IIIa) become exposed on the surface. Binding of fibrinogen molecules to GPIIb/IIIa receptors on adjacent platelets causes platelets aggregation.

The activated platelets release ADP and TxA2 and so a self-sustaining reaction is generated leading to the formation of a platelets plug. Thrombin generated from activation of coagulation system is a potent platelets - aggregating agent and also converts fibrinogen to fibrin. Fibrin and aggregated mass of platelets at the site of injury constitute the haemostatic plug. [25]

2.4.4.6.4 platelets procoagulant activity When platelets are activated, negatively charged phospholipids (phosphotidylserine and phosphatidylinositol) located in the inner half of the lipid bilayer become exposed on the outer surface. These phospholipids play active role in coagulation by providing surface for interaction of some coagulation factors. Critical coagulation reactions for which activated platelets provide negatively charged phospholipid (PL) surface. platelets may play a role in the activation

of F XII in the presence of ADP and kallikrein. platelets also can directly activate F XI independent of F XII. This may explain the absence of bleeding diathesis in persons with F XII deficiency [25]

2.5 D-Dimer

D-dimer, a degradation product of cross-linked fibrin formed during activation of the coagulation system, is commonly used to exclude thromboembolic disease in outpatients suspected of having deep venous thrombosis (DVT) and pulmonary embolism (PE). [34] DVT and PE is relatively common and can cause sudden, fatal embolic events in the pulmonary arteries and other regions.[35] Measurement of the D-Dimer level in plasma has been used as a screening strategy for subclinical DVT. A systematic review reported that a normal range of a highly sensitive D-dimer level accurately ruled out DVT in patients classified as having a low or moderate clinical probability of DVT. The DVT is a high-risk factor for the stroke because of advanced age, hemiplegia, and coagulation disorders, and DVT can cause paradoxical embolic stroke via a right-toleft shunt. Thus, it is important to monitor the level of D-Dimer the incidence and characteristics of DVT in acute stroke patients.[36] The Plasma D-dimer level has proven to be useful for DVT screening in chronic stroke patients undergoing rehabilitation .National and international scientific organizations have suggested the use of these markers when implementing new diagnostic strategies in patients with coronary syndrome. Since D-Dimer is well known to be an important prognostic indicator of heart diseases, its most definitive role is on monitoring post-treatment clinical status and the post therapeutic evaluation of patients.[36] Fibrinogen is a soluble plasma glycoprotein that is transformed into highly self-adhesive fibrin monomers after thrombin cleavage.. In brief, in the first step of D-dimer formation, thrombin cleavage exposes a previously cryptic polymerization site on fibrinogen that promotes the binding of either another fibrinogen or a monomeric fibrin molecule. Fibrin monomers then bind to one

another in an overlapping manner to form 2 molecule thick protofibrils Plasma remains fluid until 25% to 30% of plasma fibrinogen is cleaved by thrombin, allowing time for fibrin to polymerize while simultaneously promoting thrombin activation of plasma factor XIII. Thrombin remains associated with fibrin, and as additional fibrin molecules polymerize, it activates plasma factor XIII bound to fibrinogen. The complex between soluble fibrin polymers, thrombin, and plasma factor XIII promotes the formation of factor XIIIa before a fibrin gel is detected. In the second step of D-dimer formation, factor XIIIa covalently cross links fibrin monomers via intermolecular isopeptide bonds formed between lysine and glutamine residues within the soluble protofibrils and the insoluble fibrin gel.

D-dimer antigen remains undetectable until it is released from crosslinked fibrin by the action of plasmin. In the final step of D-dimer formation, plasmin formed on the fibrin surface by plasminogen activation cleaves substrate fibrin at specific sites fibrin degradation products are produced in a wide variety of molecular weights, including the terminal degradation products of crosslinked fibrin containing D-dimer and fragment E complex . It is uncommon to detect circulating terminal fibrin degradation products (D-dimer–E complex) in human plasma, whereas soluble high-molecular-weight fragments that contain the “D-dimer antigen” are present in patients with DIC and other thrombotic disorders. These fragments may be derived from soluble fibrin before it has been incorporated into a fibrin gel,or alternatively may be derived from high-molecular-weight complexesreleased from an insoluble clot . [37]

2.6 Previous study

Isaac et al January, 2014 studied **the effect of hormonal contraceptives on platelets count** of women in Sokoto State North Western Nigeria. their results indicates the mean platelets count was marginally higher among subjects on hormonal contraceptives compared to controls, this difference however was not statistically significant (p = 0.851). They observed a negative correlation between age of HCs users and platelets count (p=0.03). The mean platelets count was significantly lower among long-term hormonal contraceptives users compared to short-term users (p=0.05). This study indicates that early introduction of third generation hormonal contraception can produce an initial increase in the platelets count and that long-term use of hormonal contraceptives is associated with a reduction in platelets count. [1]

Abdalla TM, AAY Kordofani *, AAH Nimir 2008 their studied **in Haemostatic studies in Sudanese women on oral contraceptive pills.**

The study aimed at detection of haemostatic derangements in Sudanese women using contraceptive pills containing oestrogen and progesterone (combined pills) or progesterone only (mini pills) for short and long duration. Their result indicates the mean values for platelets is similar in controls and users of mini pills but were significantly different in users of combined pills. Also their result shows that platelets count and fibrinogen levels were significantly higher in users of combined pills. ($p < 0.05$).[3]

Sajida S. H et.al 2006*;* their studies were in The **Effect of Oral Contraceptive Pills on Haematological Indices** Running Title: Haematological Parameters in Oral Contraceptive Users Seventy, Case-series study was done in family planning clinic at Al-Batool Teaching Hospital. four women using combined oral contraceptive pills were investigated of platelets count using coulter counter. There was a significant effect of OC use on platelets count in oral contraceptive users as compared to controls. [4]

Samsunnahar et al in 2014, studied **Assessment of Coagulation Disorder in Women Taking Oral Contraceptives**. Their result showed The mean total count of platelets level was significantly higher (P<0.001) in contraceptive user group.[5]

Israa S. Abbas, et al, studied on **The Effect of Combined Oral Contraceptive Pills on Factor VII Activity and D-dimer Level in Healthy and Obese Women**, this study was started on November 2014 and completed on March 2015, and included 50 females attended at Baghdad Teaching Hospital.

The hemostatic parameters done for them included the prothrombin time, activated partial thromboplastin time, factor VII, D-dimer level, and platelets count,the result showed significant increase in D-dimer levels in the non-obese and obese groups compared to the control (P= 0.029 and 0.038, respectively). The platelets count was significantly increased only in the obese group compared to the control (P = 0.027) [38]

Meijers JC et al. in 2000, studied of **Increased fibrinolytic activity during use of oral contraceptives is counteracted by an enhanced factor XI-independent down regulation of fibrinolysis*:*** fibrinolytic parameters was investigated in a cycle-controlled cross-over study in which 28 non-OC using women were randomly prescribed either a representative of the and who switched OC after a two month wash out period. The D-dimer level was significantly increased (by 30 to 80%).[39]

Klipping C et al. studied **Hemostatic Effects of a Novel Estradiol-Based Oral Contraceptive.** Their results indicates A significantly (p = 0.01) smaller increase in D-dimer levels .[40]

Ahmed J. AL-Husaynee* Muna A. Kashmoola, were studied was **Effect Of Compined Oral Contraceptive Pills On Some Haemostatic Parameters.** A prospective clinico- haematological study was carried out in private lab in Iraq. Most important haemostatic parameters including platelets

count (PC) and D-dimer were done for them. Their results indicates was a significant increase in the mean values of platelets count with ($P < 0.05$), D-dimer was significantly positive in users than non users ($P<0.001$). [41]

Chapter Three

Materials and Method

3: Material and Method

3.1 Study Design

This cross sectional study was conducted during the period from April to December 2018 that aimed to detect the effect of oral contraceptive on platelets count and D-dimer level in women in Shendi Town.

3.2 Study Area

Shendi is located in the River Nile in Sudan at an altitudeof 360 meters [1181 feet] above sea level. It is about 150kilometers [93 miles] north-east and 45kilometers [29.9 miles] from the ancient meroe ruins.It is considerd one of the most important cities in the north of Sudan in terms of its location between the north and north east of the sudan in the capital in central Sudan, its proximity to the urban communities in north east Sudan.

3.3 Study Population

The study target women who use oral contraceptive in Shendi Town.

3.4 Inclusion Criteria

All adult and consenting women on oral contraceptives for at least three months were included in the study. Control group include non- pregnant and non-users of oral contraceptives.

3.5 Exclusions Criteria

All women on oral contraceptives for less than three months, non-oral contraceptives women. Pregnant women and women with underlying disease such as history of heart, liver and kidney disease, hypertensive, diabetes mellitus also excluded from any endocrine disease, hormone replacement therapy, that might affect the clotting system were excluded from this study.

3.6 Sampling

A total of (50)samples collected from women on oral contraceptive pills and (30) samples collected from healthy women group not use contraceptive pills as control group.

3.7 Ethical consideration

This study was approved by the faculty of graduate studies, Shendi university. The laboratory testing and demographic data was collected after obtaining informed consent from the study participants.

3.8 Data collection tools

The Data was collected by using self-administrated direct interview using coded questionnaire which specifically designed to obtain information that helped in study.

3.9 Blood Sampling

Under aseptic precautions 5ml venous blood was drawn by using venipuncture and transferred into 2 separate test tubes containing 3.8% trisodium citrate and EDTA respectively, after the blood added to the anticoagulants should gently mix. Then the samples immediately were sending to the hematology laboratory.

3.10 Methods

3.10.1 platelets counts were done by using Mindray Haematology Analyzer (Mindray bc-3000):

3. 10.1.1 Principle

Blood cells can be broadly divided into three categories .red blood cells, White blood cells and platelets . 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 a solution; this method measures the changes in electrical resistance that occurs when blood cells pass through detection aperture. This instrument performs haematology analyses according to the RF/DC detection method, Hydro Dynamic Focusing (DC Detection), and sodium lauryl sulphate (SLS) haemoglobin method. The radio frequencies and direct current (RF/DC detection method) detects the volume of blood cells by changes in direct- current resistance.

3.10.1.2 Procedure

Platelets counts were measured by using an automatic blood cell counter The assay was performed according to the instructions provided by the manufacturer. The analyzer was controlled by normal control, abnormal high and abnormal low, the EDTA blood samples were aspirated into analyzer through a sample probe, and the counting was started automatically, the results were displayed on the screen within (20) second, the print key was pressed to print out the results.

3.10.2 D-Dimder

3.10.2.1 Principle

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip. The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by Instrument for ichroma™ tests to show D-Dimer concentration in sample. The working range of ichroma™ D-Dimer test is (50– 10,000 ng/ml)

Reference Value: 500 ng/ml.

3.10.2.2 Components and reagents

Ichroma™ D-Dimer consists of ‘Cartridges’, ‘Detection Buffer Tubes’ and an ‘ID chip’.

- The cartridge contains a test strip, the membrane which has mouse monoclonal anti human D-Dimer at the test line, while streptavidin at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.

- The detection buffer contains mouse monoclonal anti human D-dimer-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is pre-dispensed in a separate tube. 25 detection buffer tubes are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment

3.10.2.3 Test procedure

1- Transfer 10 μL of sample (Human whole blood / plasma / control) using a transfer pipette to a tube containing the detection buffer.

2- Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately.)

3- Pipette out 75 μL of a sample mixture and dispense it into the sample well on the cartridge.

4- Leave the sample-loaded cartridge at room temperature for 12 minutes.

5- To scan the sample-loaded cartridge, insert it into the cartridge holder of the Instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.

6- Press 'Select' button on the Instrument for ichroma™ tests to start the scanning process.

7- Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.

8- Read the test result on the display screen of the Instrument for ichroma™ test

3.11 Data analysis

The data was analyzed using SPSS-20 and included the descriptive measures of frequency, percentages, mean and standard deviation. Pearson correlation was used to measure the relationship between two variables Pearson's

correlation coefficient was tested using t-test. Two tailed t-test was applied for testing the significance of difference of two independent sample means of the quantitative data. P value < 0.05 was considered significant.

Chapter Four

Results

4. Results

Table (4.1): Distribution of study population.

Study group	Frequency	Percent%
Case group	50	62.5%
Control group	30	37.5%
Total	80	100%

The study include 80 women (50) as case group (62.5%), 30 as control group (37.5%).

Table (4.2): Distribution of case group according to the age.

Age	Frequency	Percent%
25 – 34 years	24	48%
35 – 45 years	26	52%
Total	50	100%

The age with range of (25 – 34) years frequency is 24 (48%), and the age with range (35 – 45) years the frequency is 26 (52%)

Table (4.3): Distribution of case group according to duration of use.

Duration of use	Frequency	Percent(%)
Less than 1 year	19	38%
1-3 year	27	54%
More than 3 year	4	8%
Total	50	100%

Frequency of case group according to duration of use oral contraceptive pills in less than one year, from (1 – 3) years and more than 3 years is 19 (38%), 27 (54%), 4 (8%) respectively.

Table(4.4) Distribution of the case group according to the breastfeeding.

Breast feeding	Frequency	Percent(%)
Yes	23	46%
No	27	54%
Total	50	100%

The frequency of women who breast feeding is 23 (46%), and women not breast feeding 27 (54%).

Table (4-5): Distribution of the case group according to the number of pregnancies.

No of pregnancies	Frequency	Percent(%)
1-3	33	66%
4-6	17	34%
Total	50	100%

The frequency of women according to number of pregnancy was ranged from (1-3) and (4 – 6) is 33 (66%), 17 (34%) respectively.

Table(4-6): Mean of platelets count of study population.

Platelet × 10^9/L	Mean	Std deviation	P.vlue
Case group	315	69.2	.028
Control group	281	61.4	

The mean values of platelets count in case group is (315 × 10^9/L) and in control group the mean values of platelets is (281 × 10^9 /L) (P.value = 0.028).

Table(4-7):Mean of D-dimer level of study population.

D-dimer (ng/ml)	Mean	Std diviation	P.vlue
Case group	218	104.3	.000
Control group	128	39.2	

The mean values of D-dimer is (218 ng/ml) in case group while the mean value of D-dimer in control group is (128 ng/ml), (P.value = 0.000).

Table (4-8):Mean of platelets count and D-dimer level according to age.

Age	Platelet × 10^9/L	D-dimer ng/ml
25 – 34 years	323	196
35 – 45 years	307	238
P.value	0.153	0.443

The mean values of platelets count and D-dimer level in case group according to age in range (25 – 34), (35 – 45) is (323 × 10^9 / L), (307 × 10^9 /L) (196 ng/ml), (238 ng/ml), (P.value = 0.153, 0.443) respectively.

Table (4-9) Mean of platelets count and D-dimer level according to duration of use between the period(<1year and 1-3years).

Duration of use	Platelet × 10^9/L	D-dimer ng/ml
<1year	328	175
1-3 year	298	235
P- value	0.135	0.044

The mean values of platelets count according to duration of use OC between the period (< 1 year and 1 – 3 years), is (328 × 10^9/L), (298 × 10^9/L) respectively and P.value is (0.135).

The mean value of D-dimer according to duration of use OC between the period (<1 year and 1 – 3 years) is (175 ng/ml), (235 ng/ml) respectively with p.value (0.044).

Table (4-10) Mean of platelets count and D-dimer level according to duration of use between the period(<1year and > 3years).

Duration of use	Platelet × 10^9/L	D-dimer ng/ml
<1year	328	175
>3 year	361	304
p.value	0.443	0.034

The mean value of platelets count and D-dimer level according to duration of use OC between the period (< 1 year and > 3 years) is (328 × 10^9/L), (361 × 10^9/L), (175 ng/ml), (304 ng/ml) respectively and P.value of platelets count and D-dimer level is (0.443, 0.034) respectively.

Table (4-11) Mean of platelets count and D-dimer level according to duration of use between the period(1-3years and > 3years).

Duration of use	Platelet × 10^9/L	D-dimer ng/ml
1-3year	398	235
>3 year	361	304
P-value	0.062	0.021

The mean values of platelets count and D-dimer level according to duration of use of OC between the period (1 – 3 years, and > 3 years) is (398 × 10^9/L), (361 × 109/L), (235 ng/ml), (304 ng/ml) respectively, and p.value of platelets count and D-dimer level is (0.062, 0.021) respectively.

Table(4-12):Mean of platelets count and D-dimer level according to breast feeding.

Parameter	Breast feeding		P.vlue
	Yes	No	
Platelet(×10^9/L)	330	302	.159
D-dimer(ng/ml)	182	249	.021

The mean values of platelets count and D-dimer level in women who breast feeding (Yes) is (330 × 10^9/L), (182 ng/dl) respectively, and the mean values of platelets count and D-dimer level in women not breast feeding (No) is (302 × 10^9/L), (249 ng/ml).

Table (4-13):Mean of platelets count and D-dimer level according to number of pregnancies.

Parameters	Number of pregnancies		P.vlue
	1-3	4-6	
Platelet($\times 10^9$/L)	327	291	.078
D-dimer(ng/ml)	191	271	.009

The mean values of platelets count and D-dimer level according to number of pregnancy in range (1-3 and 4 – 6) is (327×10^9/L), (291×10^9/L), (191ng/ml, 271ng/ml) respectively with P.vlaue (0.078, 0.009) respectively.

Chapter Five

Discussion

Conclusion

Recommendations

5.1. Discussion

Oral contraceptive use is known to cause changes in the haemostatic system. Oestrogen is a known risk factor in thromboembolic disease. Combined contraceptive pills are therefore known to be associated with a higher risk of developing thrombosis [3].

The results of this study showed an increase in the mean of platelets count (315×10^9/L) in contraceptive users when compared to control group (281×10^9/L) .and demonstrated that there was significant increase in platelets count in oral contraceptive users as compared to controls group with P.value (.028) this was agreed with the finding of other investigators, Abdalla TM et al 2008 their result indicates that platelets count were significantly higher in users of combined pills. ($p < 0.05$) [3] Sajida S. H et.al 2006 found a significant effect of OC use on platelets count in oral contraceptive users as compared to controls [4] .

Also was similar to another researcher in Nigeria Isaac et al in 2014 published that the mean of platelets count was marginally higher among subjects on hormonal contraceptives compared to controls, however this difference was statistically insignificant ($P = 0.851$) [1]. Also the present of this study were agreed with study done by Samsunnahar et al in 2014 The mean (±SE) total count of platelets level was significantly higher ($P<0.001$) in contraceptive user group [5] .Also This result was the same as the previous result adopted by Al-Husaynee AJ et al. (2006) [41] whom suggested that significantly higher platelets count in COCPs users than non-users.

The outcome of the results obtained revealed the mean of D-dimer level in the oral contraceptive user (218)ng/ml were significantly higher than that in the control group (128)ng/ml and there was strong significant statistical value depicted among study population (P.value = 0.000). These results were comparable to those reached by Israa S Abbas, et al.2014 who reported significantly positive D-dimer in oral contraceptive users than non-

users(P.value = 0.029) [38]. Meijers JC et al.2000 also found significant increase of D-dimer during the use of the pills [39] . Klipping C et al.2007 (p = 0.01) [40] observed the increase in D-dimer level after 3 months of pills use in their study. The females using the combined contraceptive pills have enhanced fibrinolytic activity [41] that may explain the significantly high D-dimer levels . Also the Results of present study were in agreed with a previous study done by AL-Husaynee AJ et al [42]. who reported significantly positive D-dimer in users than non-users.

the study showed that when comparing the platelets count and D-dimer level with age there is no significant result P-value(0.433 ,0.153)respectively.

When comparing the platelets count with a duration of use the result showed no statistically significant difference was found when comparing between the period ((less than 1year and1-3 years , less than 1year and more than 3 years , 1-3 years and more than 3 years)) p-value (.153,.443,.062) respectively this result agreement with Abdalla TM et al [3] who reported. That there were not affected by duration of use since similar results were obtained in both short term and long term users.

The results of the tests conducted showed an increase in the mean of D-dimer level according to duration of use and there was statistically significant in D-dimer level when comparing between the period ((less than 1year and1-3 years, less than 1year and more than 3 years, 1-3 years and more than 3 years)) P-value (.044, .034 , .021) respectively.

The results of this study obtained demonstrated that there was no statistically significant in platelets count in oral contraceptive users when compared to breastfeeding and the number of pregnancies P-value (.159,.078) respectively. While the study showed that there was statistically significant when comparing D-dimer to breastfeeding and number of pregnancies, P-value (.021 , .009) respectively.

5.2 Conclusion

1-Significant increase in platelets count in oral contraceptive users when compared to women not use oral contraceptive.

2- Significant increase in D-dimer level in oral contraceptive users when compared to women not uses oral contraceptive.

3-Duration of oral contraceptives use has no effect on platelets count. but there was statistically significant in D-dimer level.

4-Platelet count and d-dimer level in oral contraceptive users was not effect by age .

5-This study concluded that combined oral contraceptive pills pose a real risk of developing thromboembolic disease by causing a hypercoagulable state. That may lead to enhanced procoagulant activity.

5.3 Recommendations

1-Females should be properly assessed the platelets count and D-dimer level and monitored before starting using the pills .

2- Further study should be conducted in the future to study the effect of oral contraceptive pills on haemostatic factors other than platelets count and D-dimer level.

3- Further studies with a large sample size for each type of oral contraceptive should be conducted in the future.

Chapter Six

References

Appendix

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Appendix I

Shendi University

Faculty of Post Graduate Studies And Scientific Research

Questionnaire About

Assesment of Oral Contraceptive Pills Affect On platelets count And D- Dimer Level

Serial No ( )

(a) Demographic data:

- Age: ( )
- breest feeding: Yes ( ) ( )No
- Number of pregnancies: ( )

(b) Clinical Information:

- Use oral contraceptive pills: Yes ( ) ( )No
- Duration of using: ( ) month ( )years
- Type of contraceptive: ( )
- Any other disease: Yes ( ) No ( )
- If yes ( )
- Any medication: Yes ( ) No ( )

(c) Laboratory data:

- Platelet count: ( ) $\times 10^9$/L
- D-dimre level: ( ) ng/ml

Appendix II

Ichroma™ for D-dimer

Appendix III

(Mindray bc-3000) for CBC

Appendix IV

إقـــرار بالموافقـــة

الاسم:----------

العمر:---------- العنوان:----------

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة تأثير موانع الحمـــل الفموية على عدد الصفائح الدموية ومستوى دي دايمر.

الباحثة: ياسمين محجوب عبد الله

بعد أن شرحت لي بأنه لا يترتب عليه أي أذى جسدي أو نفسي واعلم أن المشاركة في هذا البحث لن تؤثر بأي حال من الأحوال في الرعاية الطبية التي أتلقاها كما أنه يحـــق لي بدون إبداء أسباب الانسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشراف:

د. أم كلثوم عثمان حمد

التوقيع: ---------- التاريخ:----------

Appendix V

Document No. : INS-DD-EN (Rev. 7)
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bdiTech

Cardiac

ichroma™ D-Dimer

INTENDED USE

ichroma™ D-Dimer is a fluorescence immunoassay (FIA) for the quantitative determination of D-Dimer in human whole blood / plasma. It is useful as an aid in management and monitoring of post therapeutic evaluation of thromboembolic disease patients.

For in vitro diagnostic use only.

INTRODUCTION

D-dimer, a degradation product of cross-linked fibrin formed during activation of the coagulation system, is commonly used to exclude thromboembolic disease in outpatients suspected of having deep venous thrombosis (DVT) and pulmonaryembolism (PE).[1] DVT and PE is relatively common and can cause sudden, fatal embolic events in the pulmonary arteries and other regions. [2,3]

Measurement of the D-Dimer level in plasma has been used as a screening strategy for subclinical DVT. A systematic review reported that a normal range of a highly sensitive D-dimer level accurately ruled out DVT in patients classified as having a low or moderate clinical probability of DVT. The DVT is a high-risk factor for the stroke because of advanced age, hemiplegia, and coagulation disorders, and DVT can cause paradoxical embolic stroke via a right-toleft shunt. Thus, it is important to monitor the level of D-Dimer the incidence and characteristics of DVT in acute stroke patients.[4-7] The Plasma D-dimer level has proven to be useful for DVT screening in chronic stroke patients undergoing rehabilitation.[8-10] National and International scientific organizations have suggested the use of these markers when implementing new diagnostic strategies in patients with coronary syndrome. Since D-Dimer is well known to be an important prognostic indicator of heart diseases, its most definitive role is on monitoring post-treatment clinical status and the post therapeutic evaluation of patients.

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody, which is processed by Instrument for ichroma™ tests to show D-Dimer concentration in sample.

COMPONENTS

ichroma™ D-Dimer consists of 'Cartridge', 'Detection Buffer Tube' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has mouse monoclonal anti human D-Dimer at the test line, while streptavidin at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains mouse monoclonal anti human D-Dimer-fluorescence conjugate, biotin-BSA-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is pre-dispensed in a separate tube. 25 detection buffer tubes are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip and detection buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge. After a single use, both detection buffer tube and cartridge should be discarded.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Do not keep the sample in a freezer, which could affect the test value of D-Dimer. Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ D-Dimer** as well as the Instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that Instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ D-Dimer** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ D-Dimer** should be used only in conjunction with Instrument for ichroma™ tests.
  - Any anticoagulants other than sodium citrate should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4 - 30°C.
- The detection buffer dispensed in a tube is stable for 20 months if stored at 2 - 8°C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances