Sudan University of Science and Technology

College of Graduate Studies

Effect of Hormonal Contraceptives on Platelet Count and Indices

تاثير موانع الحمل الهرمونية على عدد الصفائح الدموية ومعاملاتها

A Dissertation Submitted in Partial Fulfillment of the Requirements of M.Sc in Medical Laboratory Science (Hematology and Immunohematology)

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2018

بسم الله الرحمن الرحيم

قال تعالى :

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ ﴿٣٢﴾

سورة البقرة الآية 32

Dedication

To father and mother

To my husband

To all my teachers

To my brothers and sisters

And for all peoples who helped me in this research.

Acknowledgement

All thanks to Allah from the start live to now who give me health, power, and support.

I would like to thank all of those helped and supported me to learning and improvement with information specially my supervisor: Elshazali Widaa Ali

I would like to thank all the family of Sudan University for Science and Technology and laboratory staff at Al Shahida Nada center.

Abstract

Background: Contraceptive is used to prevent pregnancy by interfering with normal process of ovulation, fertilization, and implantation. The contraceptive was reported to has an effect on platelet count, indices and coagulation.

Objective: this study aimed to evaluate the effect of hormonal contraceptives on platelet count and indices.

Materials and methods: This was a case-control study, conducted to evaluate the platelet count, MPV, and PDW among 49 women using contraceptives (pill, mini pill, injectable and implant) and age matched 50 women not using contraceptive as a control group. The study was conducted at Al-Shahida Nada Center in Khartom state, in the period from January to March 2017. 2 ml of venous whole blood sample were collected into EDTA container and complete blood count was performed using automated hematology analyzer (Sysmex KX21N). Age of women were using contraceptive range from 15-45 years (Mean±SD33.2±5.3) and matched age of women not using contraceptives (Mean±SD32.9±8). Thirty seven (75.51%) of women were using contraceptive pill, Four (8.16%) using contraceptive mini pill, Six (12.24%) using injectable contraceptive, and Two (4.08%) using implant contraceptive. The duration of contraceptive use was range from 1-126 months.

Results: The comparison of platelet count, MPV and PDW in women using contraceptives and those not using contraceptives showed that, mean platelet count was lower in women using contraceptives than those not using contraceptives but the difference was not statistically significant (Mean±SD:269.5±92.3 and 338.2±298.7 respectively, P.*value* = 0.10). MPV was significantly higher in women using contraceptives than those

not using contraceptives (Mean±SD:11.6±2.7vs9.3±1.6, P.*value =0.00*) while PDW was significantly lower in women using contraceptive than those not using contraceptive (Mean±SD:12.6±2.5 vs14.5±2.0, P.*value=0.00*).

MPV was found significantly higher in women using contraceptive pill compared to other types (P. *value* =0.0) and PDW was significantly higher in women using minipill and injectable contraceptives than other types. No statistically significant correlation was found between platelet count and indices and duration of contraceptive use.

Conclusion: Hormonal contraceptives have no effect on platelet count while MPV was a significantly higher of women using contraceptives pill compared with other types of contraceptives

مستخلص الدراسة

الخلفية: موانع الحمل تستخدم لمنع حدوث الحمل بواسطة منع عمليات التبويض , التخصيب او التزريع. موانع الحمل تؤثر على عدد الصفائح الدموية , ومؤشراتها.

الاهداف: هذه الدراسة لتقييم تاثير موانع الحمل على عدد الصفائح الدموية ومعاملاتها.

المواد و الطرق: هذه دراسة الحالات والشواهد لتقيم عدد الصفائح الدموية, متوسط حجمها و توزيعها في 49 امراة يستخدمن موانع الحمل (الحبوب , الحقن , الشريحة) و 50 امراة مماثلات لهن في العمرلا يستخدمن موانع الحمل كمجموعة ضابطة .الدراسة اجريت في مركزالشهيدة نديفي محلية الخرطوم في الفترة من يناير الى مارس 2017. جمعت عينة دموية بمقدار 2 مل في مضاد للتجلط و اجري تعداد الدم الكامل بواسطة محلل الدم الذاتي(سيسمكس ك اكس 21 ن). النساء اللاتي يستخدمن موانع الحمل تتراوح بين 15-45 سنة (المتوسط±الانحراف المعياري: 33,2±5,3) وكذلك اعمار النساء اللاتي لا يستخدمن موانع الحمل (المتوسط±الانحراف المعياري:32,9±8) . 37 (75,51%) يستخدمن حبوب منع الحمل ثنائية الهرمون, اربعة (8,16%) يستخدمن حبوب منع الحمل احادية الهرمون , ستة(12,24%) يستخدمن الحقن و اثنين (4,08) يستخدمن الشريحة. مدة استخدامهن للموانع تتراوح بين 1-126 شهر.

النتائج: بمقارنة عدد الصفائح الدموية , حجمها و توزيعها لدى النساء اللاتي يستخدمن موانع الحمل و اللاتي لا يستخدمنها, متوسط عدد الصفائح الدموية اقل لدى النساء اللاتي يستخدمن الموانع من اللاتي لا يستخدمنها لكن الاختلاف ليس له دلالة احصائية (المتوسط±الانحراف المعياري:269,5±92,3 و 338,2±298,7 على التوالي, الدلالة الاحصائية=0,10). متوسط حجم الصفائح الدموية ذو دلالة احصائية اعلى لدى النساء اللاتي يستخدمن موانع الحمل من النساء اللاتي لا يستخدمن موانع الحمل(المتوسط±الانحراف المعياري:11,6±2,7 مقابل 9,3±1,6, الدلالة الاحصائية=0,0) بينما توزيع الصفائح الدموية ذو دلالة احصائية اقل لدى النساء اللاتي يستخدمن موانع الحمل من النساء اللاتي لا يستخدمن موانع الحمل (المتوسط±الانحراف المعياري=12,6±2,5 مقابل 14,5±2,0, الدلالة الاحصائية=0,0).

وكان متوسط حجم الصفائح الدموية اعلى لدى النساء اللاتي يستخدمن حبوب منع الحمل ثنائية الهرمون بالمقارنة مع الانواع الاخرى (الدلالة الاحصائية=0,0) و كان توزيع الصفائح الدموية اعلى لدى النساء اللاتي يستخدمن حبوب منع الحمل احادية الهرمون و الحقن من الانواع الاخرى. ليس هناك ارتباط احصائي بين عددالصفائح الدموية ومؤشراتها و مدة استخدام مانع الحمل.

الخاتمة :موانع الحمل الهرمونية ليس لها تاثير على عدد الصفائح الدموية بينما متوسط حجم الصفائح الدموية ذو دلالة احصائية اعلى لدى النساء اللاتي يستخدمن حبوب منع الحمل بالمقارنة مع الانواع الاخرى.

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Introduction and Literature Review

Chapter One

Introduction and Literature Review

1.1 Introduction

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 method such as abstinence around the time of ovulation. It used to limit family size and space births (Kirch, 2008).

Hormonal methods 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 (Smltzar *et al.*, 2010).

The platelet count is of value for assessing bleeding disorders (Au Buchon, 1996). This test is indicated when the estimated platelet count on a blood smear appears abnormal. It is also part of a coagulation profile or workup (Fischbach, 2003)

Platelet indices are useful marker for the diagnosis of thromboembolic diseases. They include mean platelet volume, platelet distribution width, plateletcrit, platelet larger cell ratio (Cavanaugh, 2003).

The use of hormonal contraceptive has been associated with increased risk of thrombosis (Lidegaard *et al.*, 2009).

1.2 Literature review

1.2.1 Blood

Blood is composed of fluid called plasma and cells. Plasma derives from the intestines and lymphatic systems. The cells are red cells (erythrocytes), white cells (leukocytes) and platelets (thrombocytes) (Fischbach, 2003).

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 (Monica, 2005).

1.2.1.1 Functions of blood

Blood has important transport, distribution, regulatory, and protective functions in the body.Transportation and distribution Oxygen is carried from the lungs to the tissues.This function is performed by haemoglobin .

Nutrients absorbed from the digestive tract are transported to the cells of the body for use or storage.Waste products of metabolism are transported from the tissues to site of excretion, e.g. carbon dioxide produced from cellular activity is carried to the lungs for excretion, and the waste products of protein metabolism are transported to the kidneys for excretion. Hormones are carried from endocrine glands to the organs where they are needed. Buffer systems in the plasma maintain the pH of

the blood between pH 7.35–7.45 and the pH in body tissues within the physiological limits required for normal cellular activity (Monica, 2005).

Proteins (particularly albumin) and salts (particularly sodium chloride) regulate plasma osmotic pressure, preventing excessive loss of fluid from the blood into tissues spaces. Blood assists in regulating the temperature of the body by absorbing and distributing heat throughout the body and to the skin surface where heat which is not required is dissipated (Monica, 2005).

When a blood vessel is damaged, platelets and blood coagulation factors interact to control blood loss. Platelets adhere to the damaged tissue and to one another and activated coagulation factors lead to the formation of fibrin and a thrombus clot which reinforce the platelet plug. Leukocytes are involved in the body's immune defences, producing antibodies in response to infection, and protecting the body from damage by viruses, bacteria, parasites, toxins and tumour cells (Monica, 2005).

1.2.1.2 Blood cell production

Hematopoiesis is the process of blood cell formation. In the adult, blood cells are manufactured in the red marrow of relatively few bones, notably the sternum, ribs, vertebral bodies, pelvic bones, and proximal portions of the humerus and the femur. In the embryo, blood cells are derived from the yolk sac mesenchyme. As the fetus develops, the liver, the spleen, and the marrow cavities of nearly all bones become active hematopoietic sites. In the newborn, hematopoiesis occurs primarily in the red marrow, which is found in most bones at that stage of development. Beginning at about age 5 years, the red marrow is gradually replaced by yellowish fat-storage cells (yellow marrow), which are inactive in the hematopoietic process. By adulthood, blood cell production normally occurs in only those bones that retain red marrow activity. Adult reticuloendothelial cells retain the potential for hematopoiesis, although in the healthy state

reserve sites are not activated. Under conditions of hematopoietic stress in later life, the liver, the spleen, and an expanded bone marrow may resume the production of blood cells. All blood cells are believed to be derived from the pluripotential stem cell, an immature cell with the capability of becoming an erythrocyte, a leukocyte, or a thrombocyte. In the adult, stem cells in hematopoietic sites undergo a series of divisions and maturational changes to form the mature cells. As they achieve the blast stage, stem cells are committed to becoming a specific type of blood cell. As the cells mature, they lose their ability to reproduce and cannot further divide to replace themselves. Thus, there is a need for continuous hematopoietic activity to replenish worn-out or damaged blood cells (Cavanough, 2003).

1.2.1.3Control of blood cell production

The formation different types of blood cell is essential for the development of normal individual. New blood cell formed from stem cell and belong to different cell lineages that induced by interleukin-3, granulocyte macrophage colony stimulating factor, erythropoietin, erythroblast enhancing factor, platelet derived groth factor, prostaglandins (Foa, 2012)

1.2.1.4 Platelet

Formed in the bone marrow by fragmentaion of megakaryocyte cytoplasm. Megacaryoblast is precursor of megacaryocyte, arises by differentiation of haemopoietic stem cell.The megacaryocyte mature by endomitotic synchronous nuclear replication, enlarging cytoplasmic volume as the number of nuclear lopes increase in multiples of two. At eight nuclear stage, the cytoplasm becomes granular and platelets are liberated. Platelet production follows formation of microvesicles in the cell cytoplasm which coalesce to form platelet demarcation membranes.

Each megacaryocyte is responsible for production about 4000 platelets. From differentiation of stem cell to production of platelets about 10 days (Hoffbrand *et al.*, 2001). Thrombopoietin is regulator of platelet production, produced by liver and kidneys. Platelets have receptors (C-MPL) for thrombopoietinand remove it from circulation. It is increases the number and rate of maturation of megacaryocytes. Interleukin-11 (IL-11) can increase the circulating platelet count (Hoffbrand *et al.*, 2001).

The normal platelet lifespan is 7-10 days. Up to one-third of the marrow output of platelet may trapped in the spleen but this rises to 90% in plenomegaly (Fischbach, 2003).

1.2.1.4.1 Platelet structure and function

The glycoproteins of the surface coat are important in plaetlet reaction of adhesion and aggregation during haemostasis. essential, manual laboratory. Adhesion to collagen by glycoprotein Ia (GPIa). Glycoprotein Ib and IIb/IIIa are important in Willebrand factor (VWF) and to vascular subendothelium. The binding site for IIb/IIIa is receptor for fibrinogen (Hoffbrand *et al*., 2001).

The plasma membrane invaginates into 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 (platelet factor 3) are importance in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa). In the platelet interior calcium, nucleotides (adenosine diphosphate (ADP) and adenosine triphosphate (ATP)) and serotonin are contained in electron-dense granules. The specific alpha granules contain a heparin antagonist, platelet-derived growth factor (PDGF), beta-thromboglobulin, fibrinogen, vWF and other clotting factors. Dense granules contain ADP, ATP, 5-hydroxytryptamine (5-HT) and calcium. Other specific organelles include lysosomes which contain

hydrolytic enzyme, and peroxisomes which contain catalase. During the release reaction, the contents of granules are discharged into open canaliculer system (Hoffbrand *et al.*, 2001).

Platelets serve two main functions to protect intact blood vessels from endothelial damage provoked by the countless microtraumas of day-to-day existence and to initiate repair through the formation of platelet plugs when blood vessel walls are damaged. When overt trauma or microtrauma damages blood vessels, platelets adhere to the altered surface. Adherence requires the presence of ionized calcium(coagulation factor IV), fibrinogen (coagulation factor I), and a protein associated with coagulation factor VIII, called von Willebrand's factor (vWF) (Cavanuogh, 2003). The process of adherence involves reversible changes in platelet shape and, usually, the release of adenosine diphosphate (ADP), adenosine triphosphate (ATP), calcium, and serotonin. With a strong enough stimulus, the next phase of platelet activity, platelet aggregation, occurs and results in the formation of a loose plug in the damaged endothelium. The platelet plug aids in controlling bleeding until a blood clot has had time to form (Cavanuogh, 2003).

1.2.1.4.2 Platelet count

The platelet count is of value for assessing bleeding disorders that occur with thrombocytopenia, uremia, liver disease, or malignancies and for monitoring the course of disease associated with bone marrow failure (Au Buchon, 1996). This test is indicated when the estimated platelet count on a blood smear appears abnormal. It is also part of a coagulation profile or workup. Normal Platelet count in adults is 140–400 × 10^3 /mm^3or 140–400 × 10^9/L and in children is 150–450 × 10^3 /mm^3or 150–450 × 10^9/L (Fischbach, 2003).

1.2.1.4.3 Clinical implications

Thrombocytosis is abnormal increase of platelet count which associated with infectious and inflammatory condations such as osteomyelitis and rheumatoid arthritis, chronic blood loss, red cell destruction, splenectomy, following recovery from marrow suppression, myeloproliferative neoplasm (Bain *et al*., 2012).

Thrombocytopenia is abnormal decrease of platelet count which associated with autoantibodies (autoimmune or idiopathic thrombocytopenic purpura), HIV infection, anticancer chemotherapy, druges such as thiazide diuretics, alcohol excess, hypersplenism,

thrombosis, disturbed renal and hepatic function and haemolytic anaemia, thrombotic thrombocytopenic purpura, pregnant women and acute leukaemia (Bain *et* al., 2012).

1.2.1.4.5 Platelet indices

Platelet indices including mean platelet volume MPV, platelet distribution width PDW and plateletcrit. They may be potential diagnostic benefit for assessing response to antiplatelet therapy in hypercoagulable or thrombosing patients. These parameters are available on many automated cell counters (Bick, 2002)

1.2.1.4.5.1Mean platelet volume(MPV)

It is machine-calculated measurement of the average size of platelets found in blood and included in blood test as part of CBC (Cavanaugh, 2003).

The mean platelet volume (MPV) is sometimes ordered in conjunction with a platelet count. The MPV indicates the uniformity of size of the platelet population. It is used for the differential diagnosis of thrombocytopenia (Fischbach, 2003; Wilson, 2008).

The MPV increases if measured in an impedance counter because of a change in volume of platelets but when measured by optical methods the MPV decreases because of a fall in refractile index of the platelets because of dilution of cytoplasmic contents (Wilson, 2008).

MPV increase with inherited disorders of platelet function may produce defects at each of different phases of platelet reactions leading to formation of haemostatic platelet plug.such as Glanzmanns disease which lead to failure of primary platelet aggregation because deficiency of membrane glycoproteins IIb and IIIa, Bernard-Soulier syndrome which platelets are larger than normal and deficiency of glycoprotein Ib, storage pool disease which platelets are larger than normal and absence of alpha granules with deficiency of their proteins. Also acquired disorders of platelet function included antiplatelet drugs, hyperglobulinaemia, myeloproliferative disease, myelodysplastic syndrome and uraemia that increase MPV (Hoffbrand *et al.*, 2001).

Normal mean platelet volume in adults is 7.4–10.4 μm^3 or fL and in children is 7.4–10.4 μm^3 or fL (Fischbach, 2003)

1.2.1.4.5.2 Platelet distribution width (PDW)

It provides information about the range of platelet size in a blood sample. (Provan and Krentz, 2002). It is directly measures variability in platelet size, change with platelet activation, and reflects the heterogeneity in platelet morphology.It is increase in the presence of platelet anisocytosis. PDW reported with reference ranging 8.3-56.6 % (Budak *et al*., 2016).

1.2.1.4.5.3 Plateletcrit (Pct)

It is the volume occupied by platelets in the blood as percentage and calculated according to formula

PCT = platelet count × MPV / 10000 (25-27)

Under physiological conditions, the amount of platelets in blood is maintained in equilibrium state by regeneration and elimination. In healthy subjects, platelet mass is closely regulated to keep it constant, while MPV is inversely related to platelet counts. Genetic and acquired factors, such as race, age, smoking, alcohol consumption, and physical activity, modify MPV and platelet count (Budak *et al*., 2016).

It is effective screening tool for platelet quantitative abnormalities.

Normal range is 0.22-0.24%.

1.2.1.4.5.4 Platelet larger cell ratio (P-LCR)

It is indicator of circulating larger platelets (> 12 fL), which is presented as percentage. The normal range is15-35% . it is used to monitor platelet activity (Budak *et al*., 2016).

1.2.1.4.6 Manual method

Whole blood are performed on EDTA anticoagulantblood obtained by standerd clean venipuncture. To discriminate platelets from red cells, counting by visual examination of diluted and lysed whole blood using a Neubauer counting chamber, which contains a precise volume of fluid (Michelson, 2012).

1.2.1.4.7 Automated method

There are several methods on analyzers for counting platelets including aperture impedance, optical scattering and fluorescence. Normal platelets give a classical log-normal volume distribution curve. Other derived platelet parameters are highly dependent on the individual technology and are influenced by the anticoagulant and delay time from sampling to analysis. All automated methods used for platelet counting must be demonstrated to be precise, show minimum fluctuation in repeated results on the same sample, and give linear results over the entire analytical range (Michelson, 2012).

1.2.1.4.8 Interfering Factors

Platelet counts normally increase at high altitudes, after strenuous exercise, trauma, or excitement and in winter. Platelet counts normally decrease before menstruation and during pregnancy. Clumping of platelets may cause falsely lowered results.Oral contraceptives cause a slight increase. Platelet counts are lower in Africans (Monica, 2005).

1.2.2 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 (Kirch, 2008).

1.2.2.1 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 (Smltzar *et al*., 2010).

1.2.2.1.1Progestin

The progestin causes the cervical mucus to thicken and become viscid and scant. These actions inhibit sperm penetration into the uterus. Also progestin impair 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 (Boulpaep and Boron, 2012).

1.2.2.1.2Estrogen

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 spermfrom entering the uterine cavity (Hatcher and Nelson 2007).

1.2.2.1.4 Combined oral contraception – pill

The pill is tablet contain two female hormones estrogen and progesterone. It is prescribed for hormonal manipulation of mensterual cycle, treatment of other menstrual-related condations. It is inhibit ovulation and increase cervical mucus consistency (Valle *et al*., 2007).

The pill can reduce incidence of benign breast disease, improvement of acne and reduce risk of uterine and ovarian cancers, anemia and pelvic infection (Smltzar *et al*., 2010).

1.2.2.1.5 Progesterone only pill – minipill

It is tablet containing female hormone progesterone, taken continuously. Used with breastfeeding women (shader *et al.*, 2006).

1.2.2.1.6 Injectable contraception

The intramascular or subcutaneous injection of Deoo-Provera (a long acting progestin) which inhibit ovulation. It is given by a health care

professional every three months. Can be used by lactation women and those with hypertention or sickle cell disease (Smltzar *et al*., 2010).

1.2.2.1.7 Implant

It is one of the long acting reversible contraceptive methods. Consist of one or more rods containing a progestin hormone that is inserted subdermally. Can be use for three years and effective for this long period. It provides contraceptive protection through suppression of ovulation and thicking of the cervical mucus (Hurd and Falcon, 2017).

1.2.2.2 Side effect

Weight gain, nausea, headache, skin change and change in mood. There are changes associated with estrogen use ,Change in coagulation factors may cpredispose certain women to intravascular clotting, change in rennin and angiotensin may affect blood pressure, raise triglysrides and high density lipoprotein, high-dose couse impairment of glucose toleranceand increase insulin resistance, increase risk of myocardial infraction, increase risk of venous thromboembolism. There are changes associated with progestin use, reduce triglysride s and high density lipoprotein, only implant associated with breast tenderness (Becker, 2001).

1.3 Previous studies

A case-control study included 43 women on hormonal contraceptive for at least three months and 20 age-matched women as a control group; They reported marginally higher mean platelet count among subjects on hormonal contraceptives compared to controls but difference was not statistically significant. They observed a negative correlation between age of hormonal contraceptive users and platelet count. The mean platelet

count was significantly lower among long-term (> 1 year) hormonal contraceptives users compared to short-term users. This study indicated that early introduction of third generation hormonal contraception can produce initial increase in platelet count and long-term use of hormonal contraceptives is associated with a reduction in platelets count (Erhabor *et al.*, 2014).

A case control study included 180 apparent healthy women, aged 18-47 years taking hormonal contraceptives in Jos, Nigeria and 100 women not taking the drugs as control. The mean platelet count showed no significant difference. Also the mean values of platelet count did not vary significantly by age of sampled population. The mean value of platelet count was slightly lower in multi-parae than tri-parae and quardi-parae (Joseph *et al.*, 2008).

Bulur *et al*, were conducted 95 women using oral contraceptives were investigated retrospectively at sixth month and examine mean platelet volume, which the result showed no difference between values (Bulur *et al.*, 2012).

A case-control study conducted for 73 healthy women (29 controls, 25 using oral contraceptives, and 19 using Norplant) 12 were on monophasic oral contraceptives while 13 were on triphasic preparation for at least 3 months. Platelet count and MPV were examined. Demographic differences were not significant among three treatment groups (Saleh *et al.*, 1995).

Prospective longitudinal study that involved 32 women out of 46 sexually active healthy. All the subjects received the single rod implant. The subjects served as their own control. The result showed, At 12 months there was statistical increase in the platelet count when compared with pre-insertion mean value. One acceptor had thrombocytopenia (Aisien *et al.*, 2010).

In this study done by Aisien *et al*, were studied prospective longitudinally 55 non-breastfeeding Norplant women. Blood collected at pretreatment and at 3, 6 and 12 months follow-up. The result showed, the mean values of platelet count showed no significant change at 3 months compared with pretreatment value. There was significant reduction in mean platelet count at 6 months and at 12 months (Aisien *et al.*, 2005).

A total of 67 women were recruited for the case-control study consisting of 47 subject and 20 age-matched controls, who were aged between 17-37 years. All haemostatic parameters were determined before and after three months of use oral contraceptive pill. The mean of platelet count were significantly increased after three months of use oral contraceptive pills (Babatunde and Olatunji, 2004).

Rationale

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 (Kirch, 2008).

The platelet play an important role in blood clotting and haemostasis. Increase or decrease of platelet count affects normal haemostasis(Monica 2005). MPV is a machine-calculated measurement of the average size of platelets found in blood (Cavanaugh, 2003). PDW is provide information about the range of platelet size in a blood sample.

(Krentz and Provan, 2002).

There are many studies reported that contraceptives have effect on platelet count and volume.

Although, CBC is performed routinely for diagnosis and monitoring of many condations but it does not performed routinely for women using contraceptives.

Abnormalities of platelet count and indices related to contraceptive use, if identified, can be used as predictor markers for hemostatic abnormalities may complicating contraceptives use.

Objectives

General objective

To study effect of hormonal contraceptives use on platelet count and indices.

Specific objectives

- To compare platelet count and indices in women using contraceptives and women not using contraceptives.
- To compare platelet count and indices according to type of contraceptive.
- To correlate duration of contraceptives use and women age with platelet count and indices.

Chapter Two

Materials and Methods

Chapter Two

Materialsand Methods

2.1 Materials

2.1.1 Study design

This was a prospective case-control study.

2.1.2 Study area

The study was conducted at AL Shahida Nada Center, Khartom state, Sudan.

2.1.3 Study duration

It was carried out during the period from January to May 2017.

2.1.4 Study population

Sudanese women using hormonal contraceptives as a case group and women not using contraceptives as a control group.

2.1.5 Inclusion criteria

Women using contraceptive pills, injectable, minipill and implants were enrolled in this study.

2.1.6 Exclusion criteria

Pregnant women, women with known haemostatic disorder, and women using anti-platelet or anticoagulants were excluded from the study.

2.1.7 Sample size

A total of 99 women were recruited for the study, 49 woman using contraceptives and 50 women not using contraceptives as control group.

2.1.8 Ethical considerations

This study was approved by of by Al-Shahida Nada Center management and Scientific Research Committee, MLS. Informed consent was taken from all women before sample collection. Women with abnormal hematological parameters was advised to visit their physicians.

2.1.9 Sample collection

Two milliliters of EDTA anticoagulant venous blood sample was collected from each participant.

2.2 Methods

2.2.1 Platelet count and indices

Platelet count and indices were performed using automated hematology analyzer (Sysmex KX21N).

2.2.2 Principle

Blood cells are diluted in a buffered electrolyte solution. A measured volume of the sample passes through an aperture tube (e.g. 100 m in diameter) between two electrodes. Interruption of the current by the non-conducting blood cells alters the electrical charge and a pulse is produced. The amplitude of each pulse is proportioned to the volume of the cell which caused it. A threshold circuit ensures only those pulses that exceed the pre-set threshold level are counted. The cell count is determined from the total number of pulses obtained from a measured

volume of blood. Analysis of the pulse heights enables mean cell volume (MPV) to be measured.

2.2.3 Data collection and analysis

Women data was collected using structured interview questionnaire and complete blood count done by automated heamatology analyzer (Sysmex KX21N, Japan) and analyzed using statistical package for social science software (SPSS), version 21.Type of statistical methods were ANOVA, T. test and Person correlation.

Chapter Three

Results

Chapter Three

Results

The platelet count, mean platelet volume (MPV) and platelet distribution width (PDW) were measured in 99 Sudanese women. 49 of them were using contraceptives and 50 were not using contraceptives.

Age of women that using contraceptives was ranged from 15-45 years (Mean±SD:33.2±5.3) and age of women that not using contraceptives was ranged from 15-45 years (Mean±SD:32.9±8).

Duration of contraceptives use was ranged from 1-126 months (Mean±SD:17.7±25.6).

Thirty seven (75.5%) of women using contraceptives were using contraceptive pill (combined of progesterone and oestrogen hormones), Four (8.2%) were using contraceptive mini pill (progesterone hormone), Six (12.2%) were using injectable contraceptive (progesterone hormone) and Two (4.1%) of women were using implant contraceptive (progesterone hormone) (Figure 3.1).

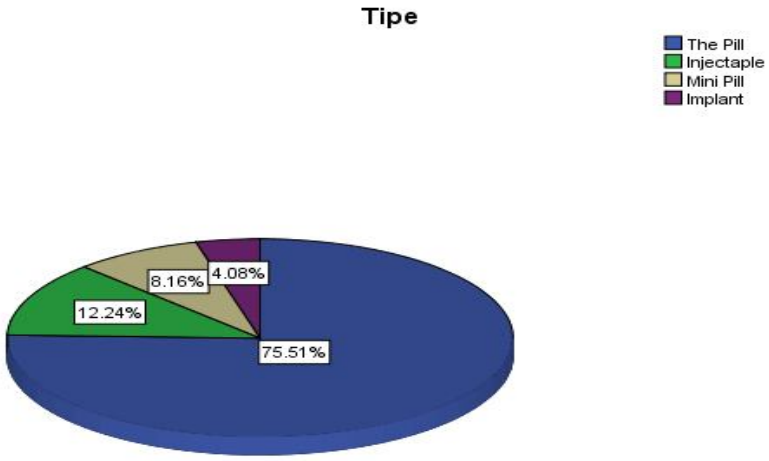

Figure 3.1 Frequencies of contraceptive types among case group

Comparison of platelet count, MPV, and PDW in women using contraceptives and those not using contraceptives showed that, mean Platelet count was lower in women using contraceptives than those not using contraceptives but the difference was not statistically significant. MPV was significantly higher in women using contraceptives while PDW was significantly lower in those using contraceptives (Table 3.1).

Table 3.1 Comparison of platelet count, MPV, and PDW in women using contraceptives and those not using contraceptives.

Platelet parameter	**Women using contraceptives**		**Women not using contraceptives**		***P. value***
	Mean	SD	Mean	SD	
Platelet count($\times 10^9$/L)	269.5	92.3	338.2	298.7	0.10
MPV(fl)	11.6	2.7	9.3	1.6	0.00
PDW(fl)	12.6	2.5	14.5	2.0	0.00

There was no statistically significant correlation between duration of contraceptives use and each of platelet count, MPV, and PDW (Table 3.2).

Table 3.2 Correlation between duration of contraceptive use and platelet count, MPV, and PDW

Parameters	**Person correlation**	***P. value***
Platelet count ($\times 10^9$/L)	-0.1	0.5
MPV (fL)	0.1	0.5
PDW (fL)	0.1	0.5

The platelet count of women using pill was (Mean±SD:285.1±94.1). The MPV of women using pill was (Mean±SD:12.2±2.6). The PDW of women us The PDW of women using pill was (Mean±SD:13.1:2.6). The platelet count of women using minipill was (Mean±SD:357.3±33.1). The MPV of women using minipill was (Mean±SD:9.4±1.4). The PDW of women using minipill was (Mean±SD:10.7±0.6). The platelet count of women using injectable contraceptive was (Mean±SD:270.3±62.7). The MPV of women using injectable contraceptive was (Mean±SD:9.1±1.9). The PDW of women using injectable contraceptive was (Mean±SD:11.1±2.4). The platelet count of women using implant contraceptive was (Mean±SD:300.5±173.2). The MPV of women using implant contraceptive was (Mean±SD:12.0±1.8). The PDW of women using implant contraceptive was (Mean±SD:12.0±1.8).

According to type of contraceptive there was statistically significant difference in mean MPV but not platelet count and PDW (Table 3.3).

Table 3.3 Comparison of platelet count, MPV, and PDW according to type of contraceptive

Platelet parameters	**Pill**		**Minipill**		**Injectable**		**Implant**		***P. value***
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Platelet count($\times 10^9$/L)	258.1	94.1	357.3	33.1	270.3	62.7	300.5	173.2	0.2
MPV (fL)	12.2	2.6	9.4	1.4	9.1	1.9	12.0	1.8	0.0
PDW (fL)	13.1	2.6	10.7	0.6	11.1	2.4	12.0	1.8	0.1

Mean MPV was statistically significant difference in women using pill (*P.value*= 0.00) compared to women using other types of contraceptives (Table 3.4).

Table 3.4 Comparison of MPV with different contraceptives

Type	**Type**	***P. value***
Pill	Injectable	0.00
	Minipill	0.00
	Implant	0.90
Injectable	Minipill	0.90
	Implant	0.20
Minipill	Implant	0.20
	Injectable	0.20

When compared mean platelet count, MPV, and PDW of women using pill, minipill, injectable, or implant with women not using contraceptive, there was statistically significant difference of mean MPV and PDW of women using pill but not of mean platelet count. Mean MPV was significantly higher in women using pill (*P.value*= 0.00) compared to women using other types of contraceptives.

There was statistically significant difference of mean PDW of women using minipill or injectable but not of mean platelet count and MPV. Mean PDW was significantly lower in women using minipill or injectable.

There was no statistically significant difference of mean platelet count , MPV and PDW of women using implant (Table 3.5).

Table 3.5 Comparison of platelet, MPV, and PDW of women using contraceptives (Pill, Minipill, Injectable, and Implant) and women not using contraceptives.

Type		Platelet count N.V:150-450 ($\times 10^9$/L)	MPV N.V:7.4-10.4 fl	PDW N.V:8.3-25fl
Pill	Mean	258.1	12.2	13.1
	S.D	94.1	2.6	2.6
	P. value	0.1	0.0	0.0
Minipill	Mean	357.3	9.4	10.7
	S.D	33.1	1.4	0.6
	P. value	0.7	1.0	0.0
Injectable	Mean	270.3	9.1	11.1
	S.D	62.7	1.9	2.4
	P. value	0.2	0.8	0.0
Implant	Mean	300.5	12.0	12.0
	S.D	173.2	1.8	1.8
	P. value	0.8	0.3	0.3
Control	Mean	338.5	9.3	14.5
	S.D	298.7	1.6	2.0

There was no statistically significant correlation between platelet count, MPV, and PDW and age of women using contraceptives (Table 4.6)

Table 4.6 Correlation between age of women using contraceptive and platelet count, MPV, and PDW.

Parameters	Person correlation	*P. value*
Platelet count ($\times 10^9$/L)	-0.1	0.5
MPV (fl)	0.1	0.6
PDW (fl)	0.0	0.8

Chapter Four

Discussion, Conclusion, and Recommendations

Chapter Four

Discussion, Conclusion, and Recommendations

4.1 Discussion

This was a case-control study conducted to evaluate the effect of contraceptives use on platelet count and indices.

In this study, there was low mean platelet count among women using contraceptive compared to controls but the difference was not statistically significant.

This finding agrees with previous studies reported that, mean platelet count was slightly lower in multi-parae than tri-parae and quardi-parae but the difference was not statistically significant (Joseph *et al*., 2008). Also Saleh reported no statistically significant difference among monophasic, triphasic and control groups (Saleh *et al*., 1995).

This finding disagree with Erhabor who reported higher mean platelet count among subjects on hormonal contraceptives compared to controls but the difference was not statistically significant (Erhabor *et al*., 2014)

In this study, MPV was significantly higher in women using contraceptives while PDW was significantly lower in those using contraceptives.

This finding disagree with Saleh who reported no statistically significant difference in MPV among monophasic , triphasic and control groups (Saleh *et al., 1995*).

In this study, there was no statistically significant correlation between duration of contraceptives use and each of platelet count, MPV, and PDW.

This finding agrees with many previous studies reported no effect for contraceptives use on mean platelet count and mean platelet volume after

a duration of three to six months (Bulur *et al.,* 2012; Saleh *et al*., 1995; Aisien *et al*., 2005; Babatunde *et al.,* 2004).

Our finding disagree with a previous study reported a significantly lower platelet count among long-term (>1 year) hormonal contraceptives users(Erhabor *et al*., 2014). Another two studies reported that, women that use implant at 12 months showed statistically significant increase platelet count (Aisien *et al*., 2010). Disagreement of our results with these results may be due difference in selection criteria as in our study duration of contraceptives use was ranged from one month to nine years.

The present study found that, according to type of contraceptive there was statistically significant difference in MPV of women using pill but not platelet count and PDW compared to control groups. Comparison of platelet count and indices in women using different contraceptives showed statistically significantly decrease of mean PDW of women using minipill or injectable but not of mean platelet count and MPV.

Our finding agrees with study by Joseph *et al* who concluded that, the mean platelet count showed no significant difference in women using injectable contraceptive (Joseph *et al*., 2008).

This finding disagree with previous studies reported that, mean platelet count showed statistically significant increase in women using implant (Aisien *et al*., 2010). Also our finding disagree with three studies all conducted that, the platelet count was significantlly increase in women using pill contraceptive (Babatunde *et al*., 2008). Also our finding is disagree with previous study reported that oral contraceptives did not change MPV values in young women (Bulur *et al.,* 2012). Disagrrement may be due to difference in selection of women that, in our study 75% of women were using pill contraceptive and the remaining 25% using the other types, while these studies conducted on women using one contraceptives type.

In this study, there was no statistically significant correlation between platelet count and indices with age of women using different types of contraceptives. This finding agrees with previous study reported that, mean platelet count did not different significantly by age of women using oral and injectable hormonal contraceptives (Joseph *et al*., 2008)

This finding disagree with previous study reported a negative correlation between age of hormonal contraceptives users and platelet count, but their study included women only using oral contraceptives for more than one year (Erhabor *et al.*, 2014).

4.2 Conclusion

- There was statistically significant lower PDW and higher MPV in women using hormonal contraceptives compared to control group.
- Platelet count was lower in women using contraceptives but the difference was not statistically significant.
- Duration of contraceptives use has no effect on platelet count or indices.
- MPV was statistically significant higher in women using pill contraceptive and mean PDW was statistically significant lower in women using minipill or injectable contraceptives.

4.3 Recommendations

- Further studies with a large sample size for each type of contraceptive should be conducted in the future.
- Women using contraceptives should be monitored by determination of platelet count, MPV and PDW periodically.
- Further study should be conducted in the future to study the effect of contraceptives on haemostatic factors other than platelets.

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Appendix

Serial No ( )

Questionaire

Sudan University of science and Technology

College of Graduate studies

Effect of Hormonal Contraceptive on Platelet Count and Indices

(a)General information:

- Age: ( )

(b)Clinical Information:

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

(c) Laboratory Investigation:

- Platelet count: ( ) × 10^9/L
- MPV: ( )fl
- PDW: ( )fl