Chapter One

Introduction and literature Review

1.1Introduction

Pregnancy outcome is influenced by many factors some of which include culture, environment, socioeconomic status and access to medical care, the hematological profile of pregnant women also has an impact on pregnancy and the outcome of the pregnancy (Ayokunle ,2011).

Pregnancy places extreme stress on the mother body and health, but her systems become prepared for the increased workload and nutritional requirements of the fetus, almost every system is involved so that is a wide range of physiological changes that associated with normal pregnancy (Hoffbrand and Moss ,2011).

Platelets are small a nucleate blood-borne particles that play a central role in blood clot formation , In areas of endothelial damage or activation of the coagulation cascade, they change shape, release their granule contents, and activate This process transforms the smooth discoid platelet into a sticky speculated particle with the ability to bind to the plasma protein fibrinogen, forming a clot , Congenital or acquired defects of platelet function are rare and usually result in minor bleeding defects. Conversely, inadvertent or excessive platelet activation is common, for example, at the site of endothelial damage, and underlies many common cardiovascular disorders, such as myocardial infarction, unstable angina, and stroke. Ant platelet agents play an important role in the management of these conditions, and a number of agents are now available to treat them (Quuinn and Fitzgerld,2005).

Platelet function is difficult to assess, with many of the assays based on platelet aggregation. The relative paucity of approaches is a major limitation to the

understanding of platelet biology, the assessment of thrombotic risk in patients, and the rational dosing of anti-platelet agents (Quuinn and Fitzgerld,2005) .

The platelet count and platelet size do not usually change during normal pregnancy, but the platelet count may fall and the mean platelet volume (MPV) may rise if pregnancy is complicated by pregnancy-associated hypertension ('toxaemia'), Pregnancy-associated thrombocytopenia of unknown mechanism occurs in a small proportion of women with an uncomplicated pregnancy (Bain, 2006).

1.2 Literature Review

1.2.1pregnancy:

Pregnancy also known as gravidity or gestation, is the time during which one or more offspring develop inside a woman ,A multiple pregnancy involve more than one offspring, such as with twins ,pregnancy can occur by sexual intercourse or assisted reproductive technology ,it usually lasts around 40 weeks from the last menstrual period(LMP) and ends in childbirth, this is just over nine lunar months ,where each month is about 29 ½ days ,when measured from conception it is about 38weeks, an embryo is developing offspring during the first eight weeks following conception, after which ,the term fetus is used until birth ,symptom of early pregnancy may include a missed period ,tender breast , nausea and vomiting ,hunger ,and frequent urination may be confined with a pregnancy test(Abman and Steven, 2011).

The mean duration of pregnancy is 38 weeks from the time of ovulation to birth, pregnancy can be difficult time for the mother because profound adaptations occur in several body systems, not only are there anatomical changes, but striking changes in metabolism and physiology occur to support the pregnancy and prepare her body for delivery and lactation (Marieb and hoehn ,2013)

Pregnancy is typically divided into three trimester ,the first trimester is from week one through12 and includes conception ,conception is when the sperm fertilizes the egg, the fertilized then travels down the fallopian tube and attaches to the inside of the uterus ,where it begins to form the fetus and placenta ,the first trimester carries the highest risk of miscarriage (natural death of embryo or fetus)(Hopkins, 2012) .

Minute ventilation is increased by 40% in the first trimester, the womb will grow to the size of lemon by eight weeks, many symptoms and discomforts of pregnancy appear in the first trimester (Cambell and Klocke, 2001).

Week 13 to 28 of the pregnancy are called the second trimester ,most women feel more energized in this period ,and begin to put on weight as the symptoms of morning sickness subside and eventually fade away, the uterus , the muscular organ that holds the developing fetus, can expand up to 20 times it is normal size during pregnancy, although the fetus begins to move and takes a recognizable human shape during the first trimester ,it is not until the second trimester that movement of the fetus , often referred to as "quickening ", can be felt, this typically happens in the fourth month, more specially in the 20th to 21st week ,or by the 19th week if the women has been pregnant before (Stacey *et al.*, 2011).

1.2.1.1 Pregnancy and changes in pregnancy:

Pregnancy is the most important physiological state for human kind since as it assure continuation of the species, pregnancy produce major physical alteration in the mother ,support the fetus as it develop the capacity of independent ,existence ,and introduce a new organ in the form of the placenta ,that provide the link between the fetus and her mother(Hylten ,1995).

1.2.1.1.1 Anatomical changes:

As Pregnancy progresses ,the female reproductive organ become increasingly vascular and engorged with blood, the enhanced vascularity increase vaginal sensitivity and sexual intensity , produced by rising levels of estrogen and prostaglandin ,the breast enlarge and engorge with blood and their areola darken ,the degree of uterine enlargement during pregnancy is remarkable , the uterus fill most of the pelvic cavity by 16 weeks ,As pregnancy continues the uterus pushes higher into the abdominal cavity ,exerting pressure on both abdominal and pelvic organs (Marieb and Hoehn, 2013).

1.2.1.2 Physiological change during pregnancy:

Maternal physiological changes in pregnancy are normal adaptation that a women undergoes during pregnancy to better accommodate the embryo or fetus, these

change such as ,cardiovascular ,hematologic ,metabolic ,renal and respiratory changes that became very important in the event of complication (Milman *et al* .,2000).

1.2.2Platelets:

1.2.2.1Historical aspects:

Platelet was described by donne, Gerber, Addison and simon. in 1872, Haymen confirmed that platelet were unique cellular elements of the blood، The origin of platelet from megakaryocytes was first described by Julius Bizzozero and was later confirmed by J.Homer Wrigh ,In 1940s and early 1950s the ultra-structure of platelets was visualized with electron microscope, In 1947 Quik and Briinkhous linked platelets to thrombin formation ,Since 1950s work has continued on the platelets biochemical and biophysical roles (Rodak ,1995).

1.2.2.2 Platelet production:

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. The precursor of the megakaryocyte-the megakaryoblast-arises by a process of differentiation from the haemopoietic stem cell ,The megakaryocyte matures by endomitotic synchronous replication (i.e. DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two, Very 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, most commonly at the eight nucleus stage, the cytoplasm becomes granular ,Mature megakaryocytes are extremely large, with an eccentric placed single lobulated nucleus and a low nuclear to cytoplasmic ratio , Platelets form by fragmentation of megakaryocyte cytoplasm, approximately each megakaryocyte giving rise to 1000-5000 platelets (Hoffbrand *et al*. , 2006) .

The time interval from differentiation of the human stem cell to the production of platelets averages approximately 10 days (Hoffbrand *et al.,* 2006).

Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys, Thrombopoietin increases the number and rate of maturation of megakaryocyte (Hoffbrand *et al.*, 2006) .

1.2.2.2.1 Megakaryoblast(MKI):

The first cell in the maturation sequence is called megakaryoblast (MKI), megakaryoblasts are typically 10-15m in diameter with high nuclear matter to cytoplasm ratio, they have a single nucleus with two to six nucleoli, the cytoplasm is scanty, blue and contain no granules, they may resemble lymphocyte or other marrow blast cells and thus cannot be accurately identified by morphology alone,

During this stage they may experience nuclear division and gain enough cytoplasm to be up to 50m in diameter, megakaryoblasts occasionally, enter the blood and travel to extra marrow sites where they mature further, occasionally, megakaryoblasts are encountered in normal peripheral blood, in patient with chronic myelocytic leukemia and other myeloproliferative disease, micro-megakaryocytes with characteristic cytoplasmic budding may be seen in the peripheral blood (Rodak, 1995).

1.2.2.2.2 promegakaryocyte(MK2):

Megakaryoblast mature into megakaryocte(MK2) which enlarges to80m, there are kinds of granules formed in the Golgi apparatus are dense, alpha and lysosomal, they are dispersed throughout the cytoplasm (Rodak 1995).

1.2.2.2.3 Basophilic megakaryocyte(MK3)

Distinct granulation and final division of the nucleus occur in the third stage called the basophilic megakaryocyte (MK3).cytoplasmic lines of demarcation begin to be released as platelets, each demarcated area consist of a membrane, cytoskeleton , system of microtubules canals and a protein of cytoplasmic granules, each area

also has a store of glycogen that will help sustain the platelet for 9to11days, the cytoplasmic fragment develops a membrane with several types of GP receptors to allow activation ,adherence, aggregation and cross linking (Rodak ,1995).

1.2.2.2.4 Megakaryocyte(MK4):

Megakaryocytes these largest of the bone marrow cells show extreme morphologic diversity (Loffler *et al*,2004).

Megakaryocytes can enter the bloodstream only in highly pathological myeloproliferative disease or acute leukemia. They are shown here in order to demonstrate thrombocyte differentiation. Megakaryocytes re side in the bone marrow and have giant, extremely hyper ploidnuclei(16times the normal number of chromosome sets on average),which buildup by endomitosism, Humoral factors regulate the increase of megakaryocytes and the release of thrombocytes when more are needed (e.g., bleeding or increased thrombocyte degradation)(Theml *et al* 2004).

1.2.2.3 Platelet structure and organelles:

The exterior coat of platelets is comprised of several glycoproteins, including integrins and leucine-rich glycoproteins. They mediate platelet adhesion and aggregation as receptors for agonists such as adenosine diphsophate (ADP), arachidonic acid, and other molecules. Electron microscopic examination shows the presence of many cytoplasmatic bodies such as α-granules and dense bodies (Munker *et al*.,2007).

Granules Three different types of storage granules related to hemostasis are present in the mature platelet. These granules are alpha granules, dense or delta granules, and lysosomes. The alpha granules are the most abundant. Alpha granules contain heparin-neutralizing platelet factor 4 (PF 4), beta-thromboglobulin, platelet-derived growth factor, platelet fibrinogen, fibronectin, von Willebrand factor (vWF), and thrombospondin. Dense bodies, named because of their appearance when viewed

by electron microscopy, contain serotonin, adenosine diphosphate (ADP), adenosine triphosphate (ATP), and calcium. Lysosomes, the third type of granule, store hydrolase enzymes. Extrusion of the contents of these storage granules requires internal, cellular contraction. Secretions from the granules are released into the open canalicular system (Turgeon ,2001).

1.2.2.4MurinePlatelets

The uniquely small size of the platelet and absence of a nucleus restricts the application of many of the modern day molecular and cell biology technologies in the dissection of platelet regulation and function , As a consequence, genetically modified mice are being increasingly used to provide a genetic means to address protein function within the platelet, although this approach must be adopted with the very real concern of species differences both with respect to role of specific proteins and vascular rheology , The former is illustrated by the nature of the receptors for one of the major platelet agonists, thrombin. Human platelets express PAR1 and PAR4 receptors, whereas murine platelets express PAR3 and PAR4 , There are also fundamental issues in rheology between human and murine vessels, as well as in platelet size and number , These are likely to have significant implications for factors such as affinities of ligand for their receptors and receptor number , Nevertheless, despite these concerns, the fundamental aspects of the processes that govern platelet activation in the mouse appear to be shared with human, and the value of murine models in analysing haemostasis and thrombosis is Immense (Hoffbrand *et al,* 2005).

1.2.2.5 Platelet function:

1-.2.2.5.1Adhesion:

If vascular injury exposes the endothelial surface and underlying collagen, platelets adhere to the s ubendothelial collagen fi bers, spread pseudopods along the surface, and clump together (aggregate). Platelet adhesion to subendothelial connective

tissues, especially collagen, occurs within 1 to 2 minutes after a break in the endothelium. Epinephrine and serotonin promote vasoconstriction. ADP increases the adhesiveness of platelets. Considerable evidence indicates that the adhesion and aggregation of platelets are mediated by the binding of large soluble macromolecules to distinct glycoprotein receptors anchored in the platelet membrane. This increase in adhesiveness causes circulating platelets to adhere to those already attached to the collagen. The result is a cohesive platelet mass that rapidly increases in size to form a platelet plug (Turgeon ,2001).

1.2.2.5.2 Aggregation

A variety of agents are capable of producing platelet aggregation, an energy-dependent process, these agents include particulate material such as collagen, proteolytic enzymes such as thrombin, and biological amines such as epinephrine and serotonin. It is believed that bridges formed by fibrinogen in the presence of calcium produce a sticky surface on platelets, This results in aggregation (Turgeon ,2001).

1.2.2.5.3 Secretion

Platelets release a number of biologically active substances upon activation, these include the contents of their α and dense granules, lysozymes, and platelet-derived microparticles. In addition, activated platelets synthesize and secrete a number of biologically active products and express the inflammatory stimulant CD40L. Platelet α-granules contain platelet-derived growth factor, P-selectin, vWF, α2 antiplasmin, G-thromboglobulin, platelet factor 4 coagulation factor V, and the adhesion molecules fibrinogen, fibronectin, and thrombospondin; the dense granules contain ADP and serotonin, The released ADP provides a feedback loop for further platelet stimulation, and serotonin enables the binding of some of the proteins released from the α-granules to a subpopulation of platelets, through an as yet undefined receptor. Platelet secretion requires the formation of soluble N-

ethylmaleimide-sensitive factor attachment protein (SNAP). Activated platelets also release membrane microparticles. These contain GPIIb-IIIa, thrombospondin, and P-selectin, enhance local thrombin generation, and induce COX-2 expression with the production of prostacyclin in monocytes and endothelial cells (Quuinn and Fitzgerld,2005).

1.2.2.5.4 Procoagulant activity:

A critical function of platelet activation is to provide a negatively charged phospholipid surface for the assembly of two multiprotein complexes that form a vital part of the coagulation cascade, namely the tenase and prothrombinase complexes , A complex of FIXa–FVIIIa on the negatively charged lipid surface converts factor X to factor Xa (tenase complex) which, in turn, forms a complex with FVa on the same surface to efficiently convert prothrombin to thrombin (prothrombinase complex) In this way, a large amount of thrombin is generated in the vicinity of the platelet surface to convert fibrinogen to fibrin and to further enhance platelet activation ,The newly generated thrombin is also able to diffuse to the surface of intact endothelial cells where it binds to thrombomodulin and activates protein C, which itself is bound to the endothelial surface via endothelial cell protein C receptor (EPCR) ,Once generated, activated protein C (APC) interacts with phosphatidlyserine on the surface of activated platelets to prevent assembly of the tenase and prothrombinase complexes through cleavage of FVa and FVIIIa   Thus, the negatively charged platelet surface also supports the protein C pathway that serves to limit the coagulation cascade (Hoffbrand *et al* 2005).

 The 'compartmentalization' of reactions to lipid surfaces in this way ensures that thrombin is generated at the place that it is required during haemostasis , The formation of the negatively charged lipid surface on activated platelets is commonly described as aminophospholipid exposure or procoagulant activity. It is

formed by the movement of phosphatidylserine from the inner to the outer leaflet of the platelet membrane (Hoffbrand *et al* 2005).

The movement of phosphatidylserine can be monitored experimentally by flow cytometry or fluorescent microscopy through the binding of annexin V, factor V or factor VIII, using either fluorescently labelled secondary antibodies or by direct labelling with a fluorescent group such as fluorescein isothiocyanate (FITC), The molecular basis of the procoagulant response, including the identity of the enzyme (or 'flipase') that promotes the translocation of phosphatidylserine across the membrane, is not established . It is recognized, however, that the response is elicited only by powerful platelet agonists and that it requires $Ca2+$entry across the plasma membrane. Platelets from four patients have been reported to be unable to undergo a procoagulant response. This clinical condition has been termed Scott syndrome and is associated with significant bleeding, although if managed, does not appear to have an effect on life expectancy (Hoffbrand *et al* 2005).

1.2.2.5.5 Late events in platelet aggregation/thrombus formation:

There is increasing evidence that stabilization of the platelet plug requires delayed intracellular signals from receptors that function only when platelets make persistent contact with other platelets , This process has been termed as the 'late-events of platelet activation' or the perpetuation phase of activation ,Several recently discovered platelet receptors have been implicated in these 'late-events' (Hoffbrand *et al,* 2005).

1.2.2.5.6 Platelets and chemotaxis:

Cell migration plays a central role in a wide variety of biological phenomena, both physiologic and pathologic, going from embryogenesis to tumour metastatization, In the inflammatory response, circulating leukocytes migrate by diapedesis across the wall of microvessels into the damaged area where they act as inflammatory cells displaying phagocytic and immune functions.,The molecular components

involved in cell migration are now largely identified and it is possible to make some generalizations across a wide spectrum of migrating cell types, including amoebae, leukocytes, fibroblasts, neurons and platelets (Gresele *et al*, 2002).

1-2-2.6 Normal hemostatic mechanism:

The hemostatic system consists of blood vessels, platelets, and the plasma coagulation system inclu ding the fibrinolytic factors and their inhibitors   When a blood vessel is injured, three mechanisms operate locally at the site of injury to control bleeding: (1) vessel wall contraction, (2) platelet adhesion and aggregation (platelet plug formation), and (3) plasmatic coagulation to form a fibrin clot, all three mechanisms are essential for normal hemostasis. Abnormal bleeding usually results from defects in one or more of these three mechanisms (Munker *et al* 2007).

1.2.2.7 Platelets disorder:

1.2.2.7.1Thrombocytopenia:

Is alow platelets count, in general thrombocytopenia can be caused by failure of marrow production (example leukemia), shortened plts lifespan (e.g Idiopathic thrombocytobenic purpura(ITP), sequestration in the spleen and dilution by massive blood transfusion (Howaed and Hamilton,2008).

ITP is adisaese characterized by immunological destriction of plts.

Acute ITPis usually seen in childhood and is tipically self-limiting, chronic ITP classically occure in young women (Howaed and Hamilton,2008).

1.2.2.7.2 Thrombocytosis:

Causes of an increase in platelet numbers include: Chronic myeloproliferative diseases, e.g. essential thrombocythaemia, polycythaemia vera, chronic myeloid leukaemia, myelofibrosis, Carcinoma (disseminated) Chronic inflammatory disease, e.g. tuberculosis, Haemorrhage, Sickle cell disease associated with a nonfunctioning spleen or after splenectomy and Iron deficiency anaemia associated with active Bleeding (Cheesbrough ,2006).

1.2.2.8 Platelates indices:

Most important parameters among them are plateletcrit (PCT), mean platelet volume (MPV) and platelet distribution width (PDW).1 Platelet activation leads to changes in platelet shape with increase in platelet swelling leading to an increase in MPV and PDW.2 Mean platelet volume (MPV) is comparable to the mean corpuscular volume (MCV) of red blood cells. Determinations of platelet size are traditionally made by microscopic measurements of platelet diameters, a method which is not readily available in routine daily practice. The automated cell counter, however, provides an MPV on each whole blood sample that is processed, which makes possible the study of platelet size in a great variety of clinical conditions. The combined interpretation of PLT, MPV and PDW appears highly useful in the differential diagnosis of thrombocytosis ((shah 2013).

1.2.2.9 Normal value of platelet indices:

Table (1.1) (Giovanetti, *et al.,* 2011)

Platelet Indices	Male	Female
PDW(fl)	9.4 - 18.1	9.8 - 18.0
MPV(fl)	8.5 - 12.4	8.1 - 12.4
P-LCR(%)	14.3 – 44	10.7 - 45.0

1.3 Pregnancy and Platelets:

The effect of pregnancy on maternal platelets count somewhat more controversial; some studies demonstrate mild decline in platelets count over course of gestation, commonly approximately 7% of pregnancies. It is typically defined as platelet count lower than 15000/ml. the most common causes of thrombocytopenia is gestational thrombocytopenia, which is mild thrombocytopenia with platelets level remaining above 70000/ml, patient who are effected usually are a symptomatic and

have no history of thrombocytopenia before pregnancy. Their platelet should retain to normal within several week following delivery (WHO 2014).

Normal Pregnancy is characterized by an increase in platelets aggregation and decreased their number in circulation with gestation,platelets lifespan decline during pregnancy .increased consumption of platelets in uteroplacental circulation has been explanation of the reduction in the number(Juan *et al*.,2011)

Normal pregnancy causes a maternal physiological hypercoagulable state in late pregnancy (Han *et al* ,2014).

The risk of venous thromboembolic events(VTE) is high during pregnancy due to both physiologic changes of pregnancy and additional impact of the inherited and acquired thrombophilia, the overall rate of venous thromboembolic events in pregnancy is 200per100.000 deliveries (Heit *et al* 2005).

In addition, the physical changes of pregnancy result in an increased thrombotic state, increased pressure on the pelvic veins from the gravid uterus and decreased flow in the lower extremities result in increased stasis, relative compression of the left iliac vein by the right iliac artery as it courses across the vessel lead to an increase of clots in the left iliac vein (Goldhaber and Tapson,2004).

Although stasis increases throughout the course of pregnancy and leg pain and swelling are more frequent during the third trimester, incidence of DVT is distributed relatively equally across trimesters (Ginsberg *et al* ,1992).

1.4 Previous study:

A previous study done to determined platelet Indices in Pregnant Women in Port Harcourt, Nigeria found that The mean platelet count for the pregnant women was 212.74 ± 63.28 x 109/L; the mean MPV 9.99 ±1.94fL; the mean PCT was $0.21 \pm 0.05\%$ and PDW was 12.68 ± 1.91fL (Pughikumo *et al*., 2015).

Other previous study done to determined blood coagulation parameters and platelet indices: changes in normal and preclamptic pregnancies and predictive values for

preeclampsia found that plts count decresed while MPVincreased(p,v<0.05)(Han., *et al* 2014).

Other Previous study done to estimated Hematological profile of normal pregnant women in Western India found that Comparison of pregnant with non-pregnant shows significant changes (p <0.05), Total platelet count reduced gradually during pregnancy. Large cross-sectional studies done in pregnancy of healthy women have shown that the platelet count does decrease during pregnancy, particularly in the third trimester (Purohit1 G *et al*.,2015).

A study of haematological profile during normal pregnancy in Chinese women , platelet (PLT) count were measured on samples of blood obtained during each pregnant trimester and the 12th and 16th week postpartum. During pregnancy, PLT count were lower. The lower reference values for PLT count during pregnancy were 61 x 109/l. (Shen et al.,2010).

Aassessment of complete blood count of Sudanese pregnant women in Port Sudan city (2012), indicated that no singnificant different between positive and negative history of abortion regard to platelets (p,v=0.06) and MPV of pregnant women with history of abortion significantly increased compared to those with no history of abortion (*p.value* 0.03) ,there were insignificant decreasd in platelet of pregnant women with previous pregnancies between 7 to 10 pregnancies compare to other group (Khalil, 2012)

A study done by Farah and Munsoor (2012) Status of iron Deficiency Anemia among Sudanese pregnant women referred to Khartoum Teaching Hospital there was significant decrease in mean of PLT in the third trimester (*Pv*<0.05).

1.5 Rationale

Pregnancy in developing countries have high mortility ratio and higher risk specially young adolescents who face higher risk of complications of death as result of pregnancy than older women(WHO2014).

Thrombocytopenia is commen occurring in approximately 8-10% of pregnancies, usually secondary to physiologic chang during gestation,namely increase in platelet activation and platelet clearance(Provan *etal* ,2010).

Pregnancy is association with profound anatomical,physiological, biochemical and endocrine change that affect multiple organ and system (Yanamandra and chandraharan 2012).

Pregnancy is a factor of hypercoagulability (pregnancy- induced hypercoagulability, as a physiologically adaptive mechanism to prevent post-partum bleeding ,howefer when combined with an additional under ling hypercoagulable states ,the risk of thrombosis or embolism may become substantial (Gresele,2008).

In this research aimed to best of our knowledge in changes of hematological parameters among pregnancy.

1.6. Objectives:

1.6.1. General Objective:

To determine of Platelets Count and t indices among normal pregnant Sudanese women at soba university hospital.

1.6.2. Specific Objectives:

- To measure platelets count and platelet indices among pregnant women and compare with non-pregnant.

- To determine the effect of, age, abortion, number of pregnancy, medication and trimesters on platelet parameter (count, indices).

Chapter Two

Materials and methods

2.1. Study design and duration:

This was analytical, case control study in a period from January to May 2016.

2.2. Study area:

The Study was conducted in Soba University Hospital. Khartoum, Sudan

2.3. Study population:

 Hundred samples were collected from Sudanese normal pregnant women and 60 samples were collected from non-pregnant women as normal control.

2.4. Inclusion criteria:

 Healthy pregnant women were included in the study.

2.5. Exclusion criteria:

 Diabetes mellitus, renal problems, other cardiovascular disease and other disease was excluded.

2.6. Data collection:

A structured questionnaire was designed to collect personal and medical information about the study group, including age, trimester, number of pregnancy, abortion, medical condition and medication used. pregnant women were selected and data collected using questionnaire which was specifically designed to obtain information that help in study.

2.7. Sample collection:

 Five ml of the Blood were collected from the superficial vein in the anticupital fossa from the study population under sterile condition and collected using the following procedure.

2.8. Method of sample collection:

2.8.1 Procedure:

Participant was set up at right position for the collection, the skin was cleaned by 70% alcohol and allowed to dry, to avoid stinging when the skin is penetrated.

A tourniquet was applied to the arm, tight sufficiently to distend the vein, but not so tightly to cause discomfort, The needle was inserted, tourniquet removed and 5ml of the blood sample were collected incontainer with EDTA anticoagulant and mix gently, Blood samples were mixed again before analyzed.

2.9. Test performed:

Platelets count and indices was done using Sysmex Automated Hematology Analyzer KX 21N series SN B 2010.

2.9.1 PDW (PLT Distribution Width):

PDW was the distribution width on 20% frequency level with the peak taken as 100%. The unit applied is fL (femto = 10-15L).

2.9.2. MPV (Mean Platelet Volume)

MPV was calculated by the machine depend on following formula:

MPV (fL) = (PCT (%) / PLT ($\times 10^3/\mu$L)) $\times$ 1000

Where PCT (%) represents the value weighted with PLT frequency and is called platelet-crit or platelet volume ratio.

2.9.3 P-LCR (Large Platelet Ratio):

This is the ratio of large platelets exceeding 12 fL discriminator and is calculated as the ratio of the particle count between the 12-fL fixed discriminator and Upper discriminator (UD) to the particle count between Lower discriminator (LD) and Upper discriminator (UD).

2.10. Principles of instrument (Sysmex):

2.10.1. Detection Principle:

This instrument performs blood cell count by DC detection method.

2.10.2. Direct Current Detection Method:

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data.

2.11. Ethical consideration:

The study was approved by Ethical Committee of the College Medical Laboratory sciences, Sudan University of Science and Technology and Written inform consent was taken from each participant.

2.12. Statistical analysis:

The results were presented as mean $\pm$standerd deviation. The Statistical analysis was performed using Statistical Package for Social Science (SPSS11.5). Means separation was performed using student t test and One-way ANOVA to determine the effect of pregnancy on platelet count and platelet indices. Data presented in form of tables and graphs *P.value* at 0.05 was considered statistically significant.

Chapter three

Results

The result show that the mean of platelet count, PDW, MPV and P-LCR in cases were (224 ±27.2, 14 ±2.7, 11.2± 8.4, 28.7±8.5) respectively and control: (276.5 ±75.1, 12.7±2, 10.1 ± 0.88, 26.5±6.6) and shows that there was no significant difference effect of pregnant on MPV and P-LCR *(p.v =0.333, p.v=0.098))* respectively ,and significant effect of pregnant on platelets count and PDW(*p.v*=0.000,*Pv*=0.001) respectively .

Table (3.1) the mean of platelet count, PDW, MPV, and P-LCR in cases and control

Variable	Mean±SD		*P.value*
	Pregnancy	Non pregnant	
Platelts count	224.5±72.7	276.5±75.1	0.000
PDW(fl)	14±2.7	12.7±2	0.001
MPV(fl)	11.2±8.4	10.1±o.88	0.333
P-LCR%	28.7±8.5	26.5±6.6	0.098
Total	100	60	

The result show the effect of history of abortion on platelets count and Indices and show significant effect of abortion on platelets count(*P.v*=0.002) and insignificant effect of abortion on PDW(*P.v*=0.392),MPV(*P.v*= 0.379) and P-LCR*(P.V*=0.302).

Table (3.2) the effect of history of abortion on platelet count and indices

Variables	Mean±SD		p.v
	Yes abortion	No abortion	
Platelets count	196.2±49.1	237.8±77.6	0.002
PDW(fl)	13.6±2.5	14.1±2.8	0.392
MPV(fl)	12.8±14.8	10.4±1.4	0.379
P-LCR%	27.4±7.4	29.3±9	0.302
Total	32	68	

The result show effect of grvidity on platelets count and Indices and show insignificant effect of on platelets count, PDW,MPV and p-LCR($P.v$=0.785, $P.v$=0.812, $P.v$ =0.436, $P.v$ = 0.750) respectively

Table (3.3) the effect of gravidity on platelet count and indices

Variables	Primgravida	Multigravida	Grandmulti gravid	p.value
Platelets count	220.41±63.94	221.56±68.32	231.91±84.58	0.785
PDW(fl)	14.15±2.69	14.11±.77	13.75±2.84	0.812
MPV(fl)	10.55±1.76	10.44±1.15	12.84±14.85	0.436
P-LCR%	29.01±8.39	29.32±8.77	27.81±8.64	0.750
Total	29	39	32	

The result show the effect of Asprin medication on platelets count and Indices and show insignificant effect of Asprin medication on platelets count , PDW,MPV and P-LCR($P.v$=0.733, $P.v$=0.815, $P.v$=0.688, $P.v$= 0.659) respectively.

Table (3.4) the effect of aspirin medication on platelet count and indices

Variable	Yes Asprin	No Asprin	*P. value*
Platelets count	219±76.9	225.6±71.6	0.733
PDW(fl)	13.9±1.6	14±2.9	0.815
MPV(fl)	10.4±0.8	11.3±9.2	0.688
P-LCR%	29.5±6.4	28.5±8.9	0.659
Total	17	83	

The result show effect of of age group on platelets count and Indices and show insignificant effect of Age group on platelets count and PDW,MPV and P-LCR*(P.v=0.872,P.v=0.934,P.v=0.368,P.v=0.702)* respectively.

Table (3.5) the effect of age group on platelet count and indices

Variable	Mean ±SD		*P.value*
	16-30 years	31-45 years	
Platelets count	223.5±68.6	225.9±78.1	0.872
PDW(fl)	13.9±2.6	14±2.8	0.934
MPV(fl)	10.4±1.4	12.3±13.2	0.368
P-LCR%	29±8.6	28.3±8.5	0.702
Total	60	40	

The results show insignificant effect of first trimester on platelet count ,PDW ,MPV and P-LCR*(P.v=0.049, p.v=0.837, p.v=0.249, pv=0.296)*,as well as in second trimester show insignificant effect on platelets ,PDW and P-LCR *(p.v=0293, p.v 0.440, p.v 0.134)* respectively except in MPV show significant effect *(p.v=0.037)*) , regard to third trimester show significant effect on plts

count,PDW and P-LCR($p.v$=0.00, pv=0.00, pv =0.005) respectively,show insignificant effect on MPV($p.v$=0.211).

Table (3.6) the effect of trimester platelet count and indices

Variable	First	Second	Third
Platelets count	219±107.54	254.58±89.9	217±62.7
PDW(fl)	12.5±1.8	12.3±1.9	14.5±2.7
MPV(fl)	9.7±0.5	9.6±1.1	11.7±9.7
P-LCR%	23.6±4.3	23.7±8.1	30.4±8.3
Total	6	19	75

Chapter four

Discussion, Conclusion and Recommendations:

4.1. Discussion:

In my study platelets count statically significant lowerd (*p.v*=0.000) and PDW significant higher than control(*p.v*=0.001),and this finding ageree with report of (Pughikumo *et al.*, 2015) in port Harcourt, Nigeria who reported that platelets count decrease (212.74±63.28) , PDW was increased with mean(12.68±1.91fl),

This due to bone marrow compensates for the rapid consumption of platelets by releasing younger and larger platelets. As well as agree with report of(Purohit1 *et al.,* 2015) that reported Total platelet count reduced gradually during pregnancy, as well as agreed with a study in China by (Shen ,2010) that reported PLT count were lower. The lower reference values for PLT count during pregnancy were 61 x 109/l.

Since normal pregnancy is characterized by an increase platelets aggregation and a decrease in number of circulating platelet with gestation , increase consumption of platelet in utroplacental circulation has been suggested to be the explanation of the reduction in the number of circulating platelets (Juan et al .,2011).

In my study history of abortion significantly lower platelets count (*pv*=0.002) and no significant different on MPV,PDW and P-LCR(*pv*=0.379, *pv*=0.392,*pv*=0.302) respectively, this disagreed with the result reported by Khalil (2012) who indicated that no significant different between positive and negative history of abortion regard to platelets (p v=0.06) and MPV of pregnant women with history of abortion significantly increased compared to those with no history of abortion (*p.value* 0.03)

In my study the third trimester there was significant lowered on plts count (*p.v*=0.00), that agree with report by (Han *et al.,* 2014) that reported platelet count

decreased during late pregnancy *(p.v>0.05)*,.in this study significant higher in PDW and P-LCR(*pv*=0.00, *pv* =0.005) ,insignificant effect on MPV (*p.v*=0.211) disagree with (Han *et al* 2014) That reported MPV increased *(p.v>0.05)*,As well as Agree with A study done by Farah and Munsoor (2012) that reported there was significant decrease in mean of PLT in the third trimester (*Pv<0.05*).

there were insignificant decreased in platelet of pregnant women with previous pregnancies between 7 to 10 pregnancies compare to other group this reported by (Khalil, 2012) *(p.v>0.05)*, agree with this study that found insignificant effect of gravidity on platelets count and indices.

4.2. Conclusion:

Pregnancy had significant decrease effect in platelet count, and significant increase PDW, had insignificant effect in MPV and P-LCR .No effect of gravidity, age and aspirin medication on platelets count and indices. History of Abortion effect on platelets count, had no effect on indices,

4.3. Recommendations:

- Platelet indices should be used as routine test for pregnant woman to evaluate the susceptibility for thrombosis and platelet activation.
- other studies should be under taken with adequate sample size and special test for platelet activation and coagulation profile for pregnant women

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A ppendix 1: Questionnaire

بسم الله الرجمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

برنامج ماجستير العلوم في المختبرات الطبية علم امراض الدم المناعة الدموية

استبيان لجمع العينات بغرض البحث

Determination of Platelet Count and Platelet Indices in Pregnant Women

ID: ———— **Name:**————————————————

Age: ———————————— **Resident** ————————————————————

Do you have pregnant?

Yes ☐ **No** ☐

Trimester:

First ☐ **second** ☐ **third** ☐

Number of pregnancy ——————————————

Do you have abortion?

Yes ☐ **No** ☐

Asprin Medication

Yes ☐ **No** ☐

Do you have any disease?

DM ☐ **Cardiovascular Disorder** ☐

other————————————————————

Tests

Platelets count ———————— **PDW**————————

MPV ————————————**P-LCR** ————————

Date: ———/ ———/——— **signature:**————————————————

Appendix2

Sysmex KX21N

Appendix 3


```
No.      4474
Date         02/02/2016
Time            12:45
Mode    WB

WBC          5.3×10³/μL
RBC          4.41×10⁶/μL
HGB          13.5g/dL
HCT          39.3%
MCV          89.1fL
MCH          30.6pg
MCHC         34.4g/dL
PLT          235×10³/μL

LYM%         33.7%
MXD%         15.6%
NEUT%        50.7%
LYM#          1.8×10³/μL
MXD#          0.8×10³/μL
NEUT#         2.7×10³/μL
RDW_SD       45.4fL
RDW_CV       13.4%
PDW          13.7fL
MPV          10.4fL
P_LCR        28.3%
```

Figure of printed result

parameter of study labeled in red

A ppendix 4

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Informed consent

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

ماجستير مختبرات طبيه

تخصص علم امراض الدم ومبحث المناعه الدمويه

الموافقه المستنيرة

قياس عدد الصفائح الدمويه ومؤشراتها لدى الحوامل السودانيات بمستشفى سوبا الجامعي

علميا قد يؤثر الحمل على تعداد الصفائح الدمويه ومؤشراتها.

تهدف هذه الدراسه لايجاد هذه العلاقه.

انا الباحث ميساء فيصل ابوالحسن من جامعة السودان للعلوم والتكنولوجيا اقوم بهذه الدراسه من اجل ايجاد العلاقه بين الحمل والصفائح الدمويه.

سوف تكون المعلومات سريه ولا تستخدم العينات الا لغرض الدراسه.

اسم المشارك......................... توقيعه.............................

رقم التلفون........................... التاريخ...........................

</div>