Chapter One

Introduction and Literature review

1.1. Introduction:

Erythropoiesis is the process of the development of red blood cell; It is increases by bone marrow as a response to anemia; it involves assessing erythrocyte production using reticulocytes count (either proportional% or absolute) and calculates Reticulocyte Production Index when anemia occurs, if bone marrow is capable of responding increased number of young un-nucleated red cell enter the circulation. These young poly chromophilic red cells released prematurely from the marrow; because of erythropoietin stimulation are called shift reticulocytes a term reflecting their premature shift from the bone marrow to peripheral blood. The reticulocyte may be significantly increased in the circulation without an actual increased in marrow red cell production (Hillman and Finch, 1985).

To use reticulocyte count as index of marrow red cell production effectiveness; it must be converted to the PRI using two mathematical corrections; the first correction determines the absolute number of circulation reticulocytes and the second compensates for the reticulocytes being shift out of the marrow early and spending alonger time in the circulation (Martin, 1988).

Ineffective erythropoiesis: ineffective erythropoiesis (RPI lower than 2.0) is caused by a defective bone marrow either from intrinsic disease, lack of essential hemopoietic factors or failure in the erythropoietic mechanism itself. The two groups of demonstrating ineffective erythropoiesis are hypoproliferative anemias and anemia secondary to maturation disorders (Hillman and Finch, 1985).

Effective erythropoiesis**:** in the case of effective erythropoiesis (RPI higher than 2.0) the marrow is in fact, the haemopoietic mechanism is functional, and the necessary for red cell production are available. The two group of anemias are usually demonstrating effective erythropoiesis are haemolytic anemia and those associated with chronic or acute blood loss (Hillman and finch, 1985).

Pregnancy is the period from conception to birth after the egg is fertilized by a sperm and then implanted in the lining of the uterus; it develops into the placenta and embryo, and later into a fetus. Pregnancy usually lasts 40 weeks, beginning from the first day of the woman's last menstrual period, and is divided into three trimesters, each lasting three months (Farlex, 2014).

A miscarriage is the loss of a fetus before the 20th week of pregnancy (webMD, 2005).

This study aimed to find out erythropoiesis abnormalities assotiated with pregnancy and miscarriage.

1.2. Literature review

1.2.1. Blood: Blood is a unique fluid compromised of many cellular elements as well as a liquid portion consisting of proteins, amino acids, carbohydrates, lipids and elements (John, 2012).

1.2.1.1. The physical states of blood:

Blood is a suspension of cells in a solute of water, water - soluble proteins, and electrolytes. The viscosity of blood = 1.1 – 1.2 centipoise. The viscosity of blood is highly influenced by red blood cell and protein concentration. Increased viscosity can occur from an elevation in the cellular components as is seen in polycythemia (increased numbers of red blood cells) and protein as seen in disorders such as multiple myeloma (elevated IgG levels) and macroglobulinemia (elevated IgM levels)]. Red cell size (smaller size increases viscosity) and the speed of blood fl ow in a given vessel also infl uence viscosity (viscosity in the aorta is much less than in a small arteriole) Blood volume averages 70 mL/kg of body weight; thus the 70 kg adult has roughly 5 liters of blood. The blood volume of an individual is approximately 7% of the total body weight. Children may have a slightly higher % (10%) blood volume to total body weight. Cellular composition of blood averages 38 – 42% in women, 40 – 44% in men; the percent volume contributed by red blood cells is called the "hematocrit " or packed cell volume. Plasma is anticoagulated blood (i.e., blood where the calcium chloride has been chelated [i.e., bound] and not available for interaction with proteins) from which the cellular components (red cells, white cells, and platelets) have been removed by centrifugation. It contains the blood coagulation proteins. Serum is the liquid in blood that has been collected without an anticoagulant. Many of the proteins have "clotted" and form a precipitate along with the cellular components of the blood. It is usually yellow in color unless the

red blood cells lyses (hemolyze) releasing free hemoglobin that gives a red color in visible light. Plasma coagulation studies can only be performed on blood that has been obtained with a proper anticoagulant (usually sodium citrate in clinical medicine) and the plasma separated from the blood cells (John, 2012).

1.2.1.2. Heamopoietic system: The hematopoietic system is characterized by turnover and replenishment throughout life. The pluripotent hematopoietic stem cell (HSC) is the progenitor of the cells in blood. The cellular elements that arise from this stem cell that circulates in blood include red blood cells, white blood cells, and platelets. Normal white blood cells in the peripheral circulation include neutrophils, monocytes, eosinophils, basophils and lymphocytes. Since the HSC also gives rise to cells of the lymphoid system, the study of hematology also includes the lymph nodes and lymph tissue. There is no specific organ for hematologic disorders and its diseases arise within the bone marrow, lymph nodes, or the intravascular compartment. The intravascular compartment where these cells circulate includes the endothelial cell lining of blood vessels and the proteins in the blood plasma. The circulating cell – endothelial cell interface and the rheological aspects of blood coursing through the intravascular compartment also influence "hematology" and its many parts. This text has been structured to introduce the trainee to the area of hematology. Since the vast majority of medical students and residents do not become hematologists, there are certain essential items that all trainees must learn about this area of medicine. The trainee will learn the physician's approach to anemia and red blood cell disorders and be able to fully evaluate a complete blood count (CBC). Screening tests for bleeding disorders for the diagnosis of an individual who has a defect in the proteins or cellular elements that prevent bleeding will be addressed. The trainee also will be exposed to the clinical, biologic, and genetic risk factors that contribute to

thrombosis. Finally, the student will be introduced to those white cell disorders that are diagnosed and treated by non - hematologists and the uncommon but serious white blood cell disorders where a hematology consultation is needed (John, 2012).

1.2.1.3. Origin of hemopoietic cells:

Hematopoiesis begins early in embryonic development. The HSC and the blood vessel lining cells or endothelial cells are thought to be derived from the same precursor cell in the aorto - gonad mesonephros (AGM) system. The common precursor to the HSC and the endothelial cell is the hematoblast. It has been proposed that this cell has the capacity to differentiate into both cell classes. The HSC is present in small numbers and retains its ability to differentiate into all blood cells as well as proliferate. In the earliest stages of embryogenesis, these cells circulate through the embryo to supply oxygen and deliver nutrients. The stem cells that arise from the AGM later in embryogenesis give rise to the blood system that seeds the liver and then the bone marrow. These cells demonstrate the ability to "travel" from the time they leave the yolk sac to populate tissues and still circulate in small numbers even in adults, a property exploited in clinical hematopoietic cell transplantation. These cells regress in the liver, kidney, and spleen, but in times of stress, they can resume blood product production as seen in myeloproliferative disorders and myelofibrosis. Under the influence of specific growth and transcription factors, cells become committed to specific lineages (John, 2012).

1.2.1.3.1. The myeloid system:

Cells of this group arise in the central marrow cavity (called the "medullary" cavity). Myeloid lineage blood cells arising elsewhere in the body are designated as "extramedullary" in origin. The myeloid system consists of the following cells: red blood cells (erythrocytes), white blood cells (neutrophils, monocytes, eosinophils, basophils) and platelets (thrombocytes). Neutrophils, eosinophils and basophils have been collectively called "granulocytes" because the presence and nature of their cytoplasmic granules define their function; however, when physicians use the term " granulocytes " , they are often referring just to neutrophils (John, 2012).

Erythrocytes (red blood cell, RBC): An erythrocyte is a specialized a nucleated cell that packages hemoglobin, the protein that is a respiratory gas transport vehicle that carries oxygen from the lungs to and carbon dioxide from tissues and back to the lungs to dispel. Erythrocytes undergo erythropoiesis whereby they mature from an early progenitor cell to the non - nucleated, biconcave disk, the erythrocyte, which with the absence of its nucleus and the flexibility of its membrane is able to bend to traverse 2– 3 micron capillaries. It is regulated by the growth factor, erythropoietin. The process of erythropoiesis takes 4 days to produce a non - nucleated biconcave disk that enters the circulation with residual RNA in its cytoplasm. A new RBC in the circulation is slightly bigger than older cells. The reticulocyte count as identifed by a special stain represents the percentage of early RBC of the total number of RBC in the circulation. Red blood cell RNA remains in the erythrocyte about 1 day, so a normal " reticulocyte count " is < 2%. The red cell life span is 120 days, and normally there are about 5 million RBC/ μ L in whole blood in adult males and 4.5 million RBC/ μ L in adult females. Old RBCs lose their energy - producing (ATP)

capacity, develop stiff membranes, and are removed from circulation by the macrophages of the mononuclear – phagocytic system of the spleen. Their hemoglobin is normally retained in the reticuloendothelial (RE) system but can be lost when there is brisk shortened red blood cell survival, i.e., hemolysis. Neutrophils: Also referred to as polymorphonuclear neutrophils PMN. In the adult, neutrophils constitute 50– 80% of the total WBC analyzed (4000– 10,000\ ^{m}L). Their functions are to phagocytize and digest bacteria, cellular debris, and dead tissue. Monocytes: Monocytes are large, mononuclear cells. They have a similar functional role to neutrophils in host defense against organisms. Once they traverse into tissues, they can differentiate into macrophages that they function to phagocytize pathogens, cellular debris and dead tissue. Eosinophils: Eosinophils are characterized by their prominent orange - reddish granules seen on Wright – Giemsa stain, usually have bilobed nuclei. Eosinophils increase in reaction to foreign protein and thus are seen in parasitic infection, allergic conditions, cancer and certain drugs. Basophils: Normally basophils are 0 – 1% of WBC differential blood count. They are often increased in patients with chronic myelogenous leukemia and other myeloproliferative disorders.Platelets (thrombocytes): Platelets bud off from the cytoplasm of the bone marrow megakaryocyte. They are a nucleated cell fragments that contain remnant mRNA. They play a central role in hemostasis as they contain many hemostatic cofactors and inhibitors in their granules (John, 2012).

1.2.1.3.2. Mononuclear phagocytic system:

The mononuclear phagocyte system consists of circulating monocytes derived from the myeloid progenitor cell in the bone marrow that migrate from the circulation into tissues and differentiate into macrophages. The mononuclear

phagocytic system is also called the reticuloendothelial (RE) system. They function as a "clean - up system" for circulating debris, microorganisms and aged, defective or antibody - coated RBC (John, 2012).

1.2.1.3.3. Lymphocyte system:

Lymphocytes are mostly in lymph nodes, but are also a large blood and bone marrow component. They are part of our adaptive immunity system. The major lymphocyte subsets are B and T cells (John, 2012).

1.2.1.4. Physiology and Pathophysiology of the Hematopoietic System:

The reason why quantitative and qualitative diagnosis based on the cellular components of the blood is so important is that blood cells are easily accessible indicators of disturbances in their organs of origin or degradation—which are much less easily accessible. Thus, disturbances in the erythrocyte, granulocyte, and thrombocyte series allow important conclusions to be drawn about bone marrow function, just as disturbances of the lymphatic cells indicate reactions or disease states of the specialized lymphopoietic organs (basically, the lymph nodes, spleen, and the diffuse lymphatic intestinal organ) (Verlarg, Stuttgart, 2002).

All blood cells derive from a common stem cell. Under the influences of local and humeral factors, stem cells differentiate into different cell lines. Erythropoiesis and thrombopoiesis proceed independently once the stem cell stage has been passed, whereas monocytopoiesis and granulocytopoiesis are quite closely "related." Lymphocytopoiesis is the most independent among the remaining cell series. Granulocytes, monocytes, and lymphocytes are collectively called leukocytes (white blood cells), a term that has been retained since the days before staining pathophysiology methods were available, when the only distinction that could be made was between erythrocytes (red blood cells) and the

rest. All these cells are eukaryotic, that is, they are made up of a nucleus, sometimes with visible nucleoli, surrounded by cytoplasm, which may include various kinds of organelles, granulations, and vacuoles. Despite the common origin of all the cells, ordinary light microscopy reveals fundamental and characteristic differences in the nuclear chromatin structure in the different cell series and their various stages of maturation. The developing cells in the granulocyte series (myeloblasts and promyelocytes), for example, showa delicate, fine "net-like" (reticular) structure. Careful microscopic examination (using fine focus adjustment to view different depth levels) reveals a detailed nuclear structure that resembles fine or coarse gravel. With progressive stages of nuclear maturation in this series (myelocytes, metamyelocytes, and band or staff cells), the chromatin condenses into bands or streaks, giving the nucleus— which at the same time is adopting a characteristic curved shape—a spotted and striped pattern (Verlarg, Stuttgart, 2002).

1.2.1.6. Hematopoiesis: Hematopoiesis is the process of the development of blood cell lineages throughout life. Hematopoiesis in necessary to replenish dying cells with new blood cells. The key role of hematopoietic cells in maintaining hematopoietic homeostasis, host immunity and tissue oxygenation requires that they are highly regulated (John, 2012).

1.2.1.6.1. The hematopoietic system: the hematopoietic system includes the elements of the blood, marrow, lymph nodes, endothelial cells, thymus and spleen that are involved in the production of all blood lineages. This system further includes cytokine - producing cells and stromal elements of the bone marrow and spleen. In human physiology, the hematopoietic system supplies various cells in the body with oxygen, contributes to the formation of blood clots

when needed, and provides protection against infection and pathogens (John, 2012).

1.2.1.6.2. Blood cells: Blood cells include red blood cells (erythrocytes, RBCs), white blood cells (leukocytes) and platelets which provide a variety of functions within the body. RBCs carry oxygen, platelets contribute to hemostasis, thrombosis and the inflammatory response, and white blood cells are involved in immunity (John, 2012).

1.2.1.6.3. Hematopoietic homeostasis: Hematopoiesis is in a delicate state of homeostasis— the process of maintaining balanced production to offsetongoing destruction of blood cells. Some cell lineages such as neutrophils only survive for several hours after release from the bone marrow into the circulation. RBCs can survive longer, lasting 60 to 120 days, and terminally differentiated lymphocytes, plasma cells, may survive for up to 20 to 30 years. Hematopoietic cell production is regulated by cytokines and growth factors and monitored by tissue sensors (tissue oxygenation for red blood cells for example). The specifi c regulators and sensors for all hematopoietic elements, however, have not been clearly elucidated (John, 2012).

1.2.1.6.4. Mature hematopoietic cells and their functions:

Mature RBCs carry hemoglobin bound oxygen to tissues and release that oxygen under the hypoxic conditions of tissues. Hemoglobin delivery of oxygen is dependent on pH and a number of other metabolic functions that alter the confirmation of the tetramer and the cooperative discharge of oxygen. RBCs also transport and release CO_2 generated from body metabolism in tissues to be expelled from the body via the lungs. Platelets contain a high concentration of proactive inflammatory, hemostatic cofactors, proangiogenic proteins, inhibitors of blood coagulation, inflammation, and fibrinolysis inside their granules. These

components are actively secreted on or about the activated platelet surface when an appropriate stimulus arises. White blood cells consist of many cell types including granulocytes (neutrophils, eosinophils and basophils), monocytes, lymphocytes and dendritic cells. Neutrophils are the most common cells. Neutrophils migrate into tissues in response to inflammation or infection where they may ingest or phagocytose particles and bacteria. These cells contain oxidases and myeloperoxidase within granules that can be activated to produce superoxide to kill ingested bacteria. Eosinophils, basophils and mast cells respond to IgE to produce acute allergic responses. Mast cells are specialized long - lived tissue resident cells similar to basophils that are the initiators of the allergic response. They secrete histamine and vasoactive proteins and recruit eosinophils and basophils in response to antigens bound to IgE. Activated eosinophils express IgE receptors and amplify the allergic response. Eosinophils also contain granules with specialized proteins important for the immune reaction to parasites. Basophils also have IgE receptors and granules containing histamine and mediate allergic inflammation. These cells are present in low numbers in the peripheral blood. Monocytes enter tissues and become resident macrophages in the lung, liver or other tissues where they also participate in the inflammatory and immune response. These cells produce a wide variety of small chemicals, such as chemokines (small peptide chemicals) and cytokines, for chemoattraction and immune modulation of all immune cells. In disease states, these cells can form clusters that result in granulomas and mediate chronic inflammation. Monocytes can also be antigen - presenting cells for the lymphoid cell population, inducing an immune response. Dendritic cells are important mediators of innate and adaptive immune responses and are the main antigen presenting cell in the body. They have been suggested to derive from both common myeloid progenitor and

common lymphoid progenitor. B lymphocytes produce antibodies in response to stimulation. The initial response results in secretion of IgM immunoglobulin. The binding specificity towards the antigen is often modest at the initiation of an immune response. Upon antigen exposure, B cells migrate to specific regions within lymph nodes termed germinal centers where the cells proliferate and generate daughter cells with higher affi nity for antigen through a mutational process referred to as somatic hypermutation. During this process the cells may switch to produce a different antibody isotype, usually IgG to carry out specific effector functions. B cells mature into long - lived plasma cells and memory B cells to maintain immunologic memory. T lymphocytes produce cells with cytotoxic, helper or suppressor functions that mediate responses to viral infections or inflammatory conditions. The cytotoxic cells are particularly important to rid the body of virally infected cells. T cells produce cytokines and chemokines that modulate most immune responses including granuloma formation and are required for B - cell antibody production (John, 2012).

1.2.1.6.5. Hematopoiesis during development and in adult:

1.2.1.6.5.1. Hematopoiesis during development: The earliest forms of blood cells are observed in the yolk sac. These cells emanate from a primitive precursor population and produce both cells with oxygen - carrying capacity and a small number of primitive lymphocytes. More definitive hematopoiesis takes place later in development in fetal liver, and during the third trimester, production is transferred to the bone marrow in the developing embryo. RBC production is unique in development because of the complex evolution in the hemoglobin locus resulting in a structured sequence of distinct hemoglobin produced during fetal life. Because of the oxygen requirements in the fetus and the absence of direct air exchange in the lung, different hemoglobins are produced during gestation. Most

significant is fetal hemoglobin or hemoglobin F, a unique tetramer that disappears normally within a few months after birth. It is the main type of hemoglobin in the fetus. It has greater oxygen - affinity than adult hemoglobin A, allowing for the extraction of oxygen from the maternal blood stream. A small percentage of adult hemoglobin or hemoglobin A appears late in gestation and becomes the dominant form within 6 months of birth, reflecting the change in oxygen requirements after birth. Interestingly, fetal hemoglobin ameliorates the disease manifestations of homozygous hemoglobin S, the cause of sickle cell anemia. For this reason, erythropoietin and hydroxycarbamide (hydroxyurea), which promotes the generation of hemoglobin F, are used to treat sickle cell anemia (John, 2012).

1.2.1.6.5.2. Hematopoiesis in adult:

In adults, hematopoiesis mainly occurs in bone marrow and thymus. Myelopoiesis (non - lymphoid) and lymphopoiesis diverge during the early stage of differentiation. The hematopoietic stem cell's first lineage commitment is to differentiate to a common myeloid progenitor or a common lymphoid progenitor. The common myeloid progenitor produces megakaryocytic, erythroid (RBC), granulocytic and monocytic lineages. The ranulocytes, the neutrophils, eosinophils and basophils, are the most phylogenetically related. Monocytes arise from a common granulocyte – monocyte progenitor. Erythropoiesis is the process of generating RBC. Thrombopoiesis refers to the formation of platelets from their precursor megakaryocytes. Erythrocytes and megakaryocytes both develop from a common precursor cell. RBC production is stimulated by the growth factor, erythropoietin. Megakaryocytes are unusual in that the cell undergoes nuclear division without cytoplasmic division; the generating cell contains a high amount of DNA content, $32n – 64n$, compared to $2n$ of normal diploid cell. Each

megakaryocyte can generate large numbers of platelets by "budding" off pieces of cytoplasm. The process is stimulated by thrombopoietin (TPO), a cytokine hormone mainly produced by liver and kidney. Since leukemias often recapitulate the normal developmental process, it is not uncommon to encounter a leukemia with both granulocyte and macrophage differentiation capable of recapitulating the common granulocyte - macrophage progenitor or less often a leukemia demonstrating erythrocyte and megakaryocyte differentiation simulating the common megakaryocyte – erythrocyte progenitor. The common lymphoid progenitor cell differentiates into B - cell, T - cell and natural killer cells. B - Lymphoid development remains localized in the bone marrow, whereas developing T cells emigrate from the bone marrow to the thymus to undergo terminal differentiation. B and T - lymphoid development requires rearrangement of the DNA in the maturing cells; the immunoglobulin locus for B cells and T - cell receptor locus for T cells. DNA recombination in the developing lymphocytes randomly combines variable, diversity and joining gene segments (VDJ) to generate antibody and T - cell receptor proteins with tremendous diversity ($> 1 \times 10^7$) to match potential antigens from a wide variety of infectious or noxious agents. The DNA rearrangement process is intimately related to T and B - cell survival and maturation as lack of effective DNA recombination results in cell death. B - lymphopoiesis occurs under the influence of IL - 7. The effects of IL - 15 and IL - 2 are important later in lymphopoiesis (John, 2012).
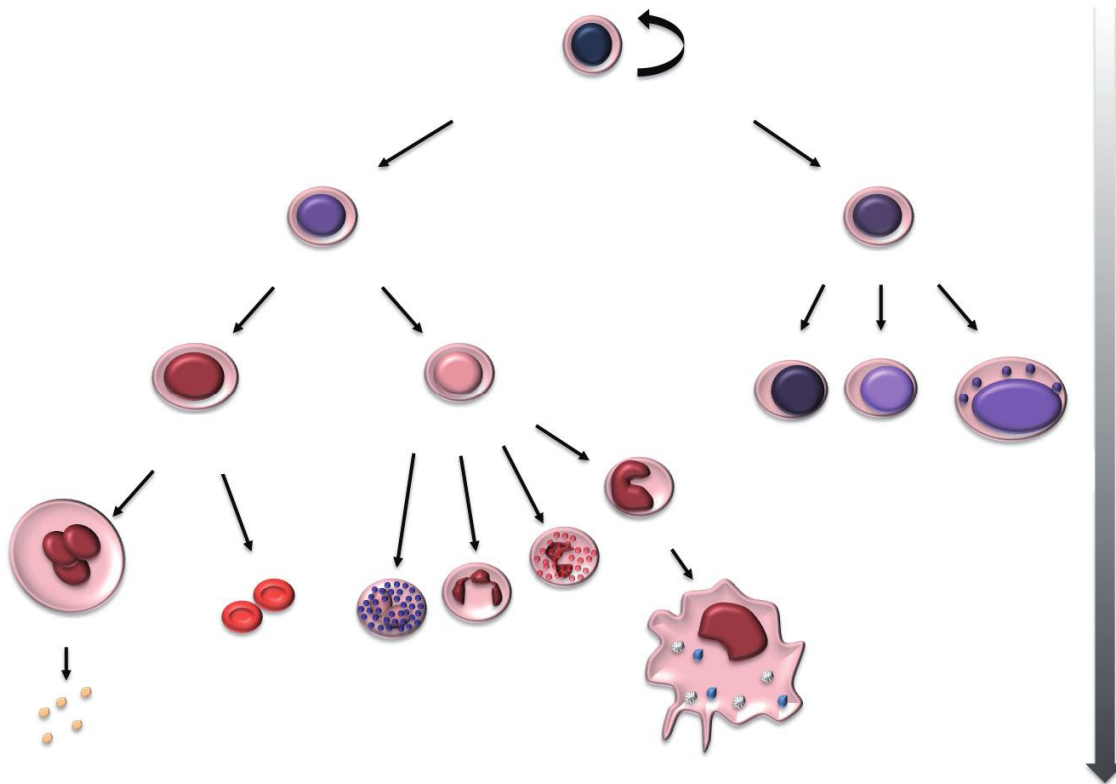
(Figure 1.1.) Show: Human hematopoiesis, Hematopoietic. Hematopoietic stem cell is one of the most primitive adult stem cells, which can self - renew and differentiate to progenitors that can give rise to all types of blood cells and platelets (John, 2012).

1.2.2. Hemoglobin: structure and function:

1.2.2.1. Structure:

Hemoglobin (Hb): is the major protein contained in mature RBCs. A hemoglobin molecule is composed of four globin chains. Each globin chain is bound to a heme moiety containing iron . Two of the globin chains are derived from the alpha - globin (α - globin) locus on chromosome 16, and the remaining two globin chains are derived from the beta - globin (β - globin) locus on chromosome 11.

Different globin chains are expressed during embryonic, fetal and postnatal/adult stages of development. Hemoglobin molecules containing different globin chains can be distinguished from one another by electrophoresis or liquid chromatography.

(a) Fetal hemoglobin (Hb F) contains two α - globin chains and two gamma - globin (γ - globin) chains. Hb F is the major hemoglobin present during the later stages of fetal development because of greater oxygen – carrying capacity.

(b) Around the time of birth, expression of γ - globin is suppressed.

(c) Beta - globin (β - globin) is the major beta - like globin chain expressed after birth and in adults, although small amounts of γ - globin and delta - globin (δ - globin) are also produced.

(d) Hemoglobin A is composed of two α - globin chains and two β – globin chains (α 2 β 2), and normally represents greater than 95% of the hemoglobin present in adult RBCs.

(e) Hemoglobin A2 (α 2 δ 2) and Hb F (α 2 γ 2) are also normally found at low levels in normal adult RBCs.

Genetic mutations in the α- globin or β- globin locus may result in the expression of an abnormal hemoglobin (hemoglobinopathy) with a different amino acid composition and aberrant migration pattern on electrophoresis. The variant hemoglobin may be functionally normal, or may have physical and/or physiologic properties that differ from a normal hemoglobin molecule.

A second category of genetic mutations in the globin loci (thalassemia): is characterized by a quantitative reduction in the synthesis of α - globin or β - globin chains, and a net reduction in the formation of hemoglobin.

1.2.2.2. Function:

1. The major physiologic role of hemoglobin is transport of oxygen from the lungs to the tissues. Oxygen binds to hemoglobin with high affinity in the oxygen rich environment of the alveolar capillary bed, and dissociates from hemoglobin in the relatively oxygen poor environment of the tissue capillary bed. The loading and unloading of oxygen from hemoglobin is facilitated by conformational changes in the hemoglobin molecule that alter its affi nity for oxygen (cooperativity).

2. Hemoglobin oxygenation is classically depicted by an oxyhemoglobin dissociation curve, where the oxygen saturation of hemoglobin is measured as a function of the partial pressure of oxygen. A convenient measure of the oxygen affi nity of hemoglobin is the partial pressure of oxygen where hemoglobin is 50% saturated (P 50). The P 50 of hemoglobin varies as a function of temperature, pH, and the intracellular concentration of 2,3- diphosphoglycerate (2,3 - DPG).

(a) Acidosis (decreased pH) and elevations in RBC 2,3 - DPG content stabilize the deoxyhemoglobin conformation, resulting in decreased affinity for oxygen, an increase in the P 50 , and a right shift in the oxyhemoglobin dissociation curve.

(b) Physiologic changes in the oxyhemoglobin dissociation curve occur as adaptive responses to anemia and/or hypoxia. Intraerythrocyte 2,3 - DPG levels are increased in individuals with chronic hypoxia or anemia and in individuals living at high altitude. The increase in 2,3 - DPG levels results in a right - shift of the oxyhemoglobin dissociation curve, and the release of a greater proportion of hemoglobin – bound oxygen in tissue capillary beds (John, 2012).

1.2.3. Red blood cell:

1.2.3.1. The mature red blood cell: A mature red blood cell assumes the shape of a biconcave disc. When viewed from above on a peripheral blood smear, it displays an area of central pallor that corresponds to the region where the upper and lower membrane surfaces of the RBC are in close proximity. The unique morphology of the red blood cell is adapted for transit through narrow capillary beds and splenic sinusoids.

1. Young, healthy red cells are highly deformable, yet rapidly return to their native shape after exiting a capillary bed.

2. Red cells become more rigid and less deformable as they age, which contributes to their senescence and elimination from the circulation in the spleen. The average life span of a red blood cell is 120 days.

1.2.3.2. The "skeleton" of the red blood cell: This is formed by a network of structural proteins that tether the membrane

lipid bilayer to the cell (Figure 3.4). Key structural proteins located in the RBC cytoskeleton include spectrin , ankyrin , and band 3 . Congenital defi ciencies in the function and/or quantity of these proteins are associated with abnormalities in RBC shape (spherocytosis or elliptocytosis) and shortened RBC survival (hemolysis).

1.2.3.3. The volume and ionic content of the RBC: The volume and ionic content are actively regulated by energy dependent pumps that traverse the membrane. These pumps depend on a constant source of adenosine triphosphate (ATP), which is generated by glycolysis within the red cell. Defects in the production of ATP are associated with loss of cell volume, increased red cell rigidity, and decreased red cell survival (John, 2012).

1.2.4. Erythropoiesis:

The mature erythrocyte is a biconcave disc with a centralpallor that occupies the middle one-third of the cell. In the mature cell, the respiratory protein, hemoglobin, performs the function of oxygen–carbon dioxide transport. Throughout the life span of the mature cell, an average of 120 days, this soft and pliable cell moves with ease through the tissue capillaries and splenic circulation. As the cell ages, cytoplasmic enzymes are catabolized, leading to increased membrane rigidity (density), phagocytosis, and destruction.

The term used to describe the process of erythrocyte production is erythropoiesis. Erythropoiesis encompasses differentiation from the hematopoietic stem cell (HSC) through the mature erythrocyte. Erythropoiesis epitomizes highly specialized cellular differentiation and gene expression. As cells progress through the stages of erythropoiesis, their potential to differentiate into lymphoid or other hematopoietic cell types is restricted. They are increasingly committed to differentiate into erythrocytes. To streamline their functional capacity, erythrocyte precursors shed most organelles and produce prodigious amounts of hemoglobin, which eventually comprises approximately 95% of the total cellular protein. Erythropoiesis is regulated partially by the combined actions of cytokine signaling pathways and transcription factors. Molecular regulators of erythropoiesis can be categorized as those committing pluripotent precursors to an erythroid fate and those regulating the differentiation of erythroid progenitors into erythrocytes. Molecular chaperones, a diverse group of proteins, are important red cell maturation. Chaperones influence all aspects of normal cellular function including signaling, transcription, cell division, and apoptosis.

Hematopoiesis begins with the development of primitive erythrocytes in the embryonic yolk sac, continues in extramedullary organs such as the liver in the

developing fetus, and is ultimately located in the red bone marrow during late fetal development, childhood, and adult life. Transport of oxygen to the tissues and transport of carbon dioxide from the tissues are accomplished by the **heme** pigment in hemoglobin, which is synthesized as the erythrocyte matures. The basic substances needed for normal erythrocyte and hemoglobin production are amino acids (proteins), iron, vitamin B12, vitamin B6, folic acid (a member of the vitamin B2 complex), and the trace minerals cobalt and nickel. In adult humans, the daily production of more than 200 billion erythrocytes requires more than 20 mg of elemental iron. The vast majority of this iron comes from the recycling of senescent erythrocytes by macrophages of the mononuclear phagocytic system; only 1 to 2 mg of the daily iron supply derives from intestinal absorption, which at a steady state is sufficient only to replace iron lost by epithelial cell sloughing and functional and dysfunctional bleeding. Abnormal erythropoiesis can result from deficiencies of any of these necessary substances. Defective erythropoiesis is frequently seen in underdeveloped countries where protein deficiencies are common. Other types of nemias can be caused by deficiencies in vitamin B12, folic acid, or iron (Mary, 2001).

1.2.4.1. Red Blood Cell Biochemistry and Physiology:

Understanding the factors that regulate RBC growth and development and the genetic and biochemical basis of RBC physiology is critical for an informed approach to the diagnosis and treatment of anemia (John, 2012).

1.2.4.1.1. Early development: Red blood cells (RBC) are normally produced in the bone marrow. The process of RBC development is called erythropoiesis . RBC are derived from pluripotent hematopoietic stem cells (HSCs), and share a common precursor (or progenitor cell) with other myeloid lineage cells including megakaryocytes, granulocytes, monocytes /macrophages, eosinophils, and

basophils. HSC arise from developing vasculature early in embryological development. Thus, inherited or acquired abnormalities in hematopoietic stem cells or myeloid progenitor cells may be associated with functional or quantitative defects in multiple types of blood cells (John, 2012).

1.2.4.1.2. Regulation of growth: The growth and maturation of RBCs from the HSC and myeloid progenitor cells is regulated by a complex interplay between genetically defined developmental programs and external signals generated by remote and/or neighboring cells (John, 2012).

1.2.4.1.3. Hematopoietic growth factors are an important class of external signals used to regulate hematopoiesis. Multiple subtypes have been identified and characterized (John, 2012).

1.2.4.1.4. Erythropoietin (EPO) is the most important growth factor regulating erythropoiesis.

(a) EPO is produced in the kidney by peritubular cells that sense tissue oxygen content. When oxygen delivery to the kidney falls (due to anemia, hypoxemia, impaired blood flow, or other causes) these renal peritubular cells rapidly increase synthesis and release of EPO.

• The normal rise in EPO associated with anemia may be blunted orabsent in patients with renal disease.

• As a result, renal disease is frequently associated with anemia and is a common indication for treatment with recombinant EPO.

(b) In response to EPO, erythroid precursors in the bone marrow are stimulated to divide and mature, resulting in increased production and release of RBC from the bone marrow.

(c) There is normally an inverse relationship between the hematocrit and plasma EPO levels. In individuals with a normal hematocrit, EPO levels are very low or

undetectable. As the hematocrit progressively declines, EPO levels increase logarithmically (John, 2012).

Extra renal organs such as the liver also secrete this substance. Ten to fifteen percent of erythropoietin production occurs in the liver, which is the primary source of erythropoietin in the unborn. This glycoprotein hormone, with a molecular weight of 46,000, stimulates erythropoiesis and can cross the placental barrier between the mother and the fetus. Erythropoietin was the fi rst human hematopoietic growth factor to be identified. The gene for erythropoietin is located on chromosome 7 (Mary, 2001).

Blood levels of erythropoietin are inversely related to tissue oxygenation. The level can increase up to 20,000 mU/mL in response to anemia or arterial hypoxemia. Erythropoietin is detectable in the plasma (normal concentration up to 20 mU/mL). The red cell mass of the body is continuously adjusted to the optimal size for its function as an oxygen carrier, by messages transmitted to the bone marrow from the oxygen sensor in the kidney. Tissue hypoxia, a decrease in the oxygen content within the tissues, produces a dramatic increase in the production of erythropoietin. A heme protein is thought to be involved in the oxygen-sensing mechanism. The messages from the sensing mechanisms are mediated by erythropoietin, are modulated by cardiovascular and renal factors, and form a key link in the feedback loop that controls red cell production. Through the action of erythropoietin, the number of hemoglobin-containing erythrocytes increases, the oxygen-carrying capacity of the blood increases, and the normal level of oxygen in the tissues can be restored (Mary, 2001).

In 1985, the erythropoietin gene was cloned and expressed. This led the way to the development of recombinant (monoclonal) human erythropoietin, which reduces transfusion dependency and increases preoperative hemoglobin in

patients whose bodies cannot respond to the need to produce erythropoietin. Recently, observations indicate that erythropoiesis-stimulating agents may be associated with serious adverse effects in patients with malignancy. Erythropoietin has its predominant effect on the committed erythroid cells, colony-forming unit-erythroid (CFU-E), promoting their proliferation and differentiation into erythroblasts. It may also stimulate the differentiation of a more primitive erythroid progenitor, the burst-forming unit-erythroid (BFU-E), in association with so-called burstpromoting activity. Erythropoietin prevents erythroid cell apoptosis. Cell divisions accompanying terminal erythroid differentiation are finely controlled by cell cycle regulators. Disruption of these terminal divisions causes erythroid cell apoptosis. In reticulocyte maturation, regulated degradation of internal organelles involves a lipoxygenase, whereas survival requires the antiapoptotic protein Bcl-x. In biochemical studies of the action of erythropoietin, it has been demonstrated that initially an increase in the production of several types of ribonucleic acid (RNA) takes place. This activity is followed by an increase in deoxyribonucleic acid (DNA) activity and protein synthesis. The number of cells at each stage before the polychromatophilic erythroblast stage is greater than at each preceding stage because of intervening cell divisions. After the polychromatophilic erythroblast stage, erythroid cells do not divide but undergo specialized maturation. Increased erythrocyte production and hemoglobin synthesis are ultimately the result (Mary, 2001).

1.2.4.2. Developmental Stages: Early Cells:

All hematopoietic cell lines are derived from an original, common pool of ancestral pluripotent stem cells. Biologic systems function at the molecular, cellular, tissue, and organismismal levels. To perform their specialized functions, highly differentiated blood cells are continuously produced by stem cells. A

combination of more than a dozen growth and stromal factors drive cells to divide asymmetrically, undergo differentiation, and carry out their end-cell functions. A simple erythrocyte, enucleated and without mitochondria, contains more than 750 proteins, ignoring posttranslational modifications. With at least a dozen types of highly specialized cells and platelets circulating a liquid phase consisting of 1,000 proteins, blood and its elements comprise a complex system. When the pluripotent stem cell, the first in a sequence of steps of cell generation and maturation, differentiates into a nonlymphoid multipotential stem cell, it can become a colony-forming unit granulocyte-erythrocytemonocyte- megakaryocyte (CFU-GEMM) depending on the presence of specific growth factors. In erythropoiesis, the CFU-GEMM differentiates into a BFU-E. The earliest cell in the erythrocyte series is the BFU-E. Like HSCs, BFU-Es are not actively proliferating. Most of these cells are in the GO/G1 phase of the cellcycle. The next step in differentiation is the formation of colony-forming units (CFU-E). CFU-Es are actively proliferating. Most are in the S phase of the cell cycle. CFU-Es produce erythroid colonies of up to 100 cells. Under the infl uence of erythropoietin, the CFU-Es undergo a programmed series of cell divisions and cell maturation, culminating in the mature erythrocyte. As CFU-Es differentiate to late-stage erythroblasts, they cease to divide and accumulate in the GO phase before enucleation. Regulated cessation of cell division preceding erythroblast enucleation is crucial for normal erythrocyte production. If it is interrupted by drugs that interfere with DNA synthesis (e.g., methotrexate) or by defi ciencies of vitamins required for DNA synthesis (e.g., folate and vitamin B12), macrocytic anemia develops. When cells differentiate into the erythroid line, the maturationalchanges are consistent with the overall nuclear and cytoplasmic

changes seen in other cell lines. However, the erythrocyte becomes an anuclear mature cell (Mary, 2001).

1.2.4.2.1. Rubriblast (Pronormoblast):

The rubriblast or pronormoblast has an overall diameter of approximately 12 to 19mm. The nuclear-to-cytoplasmic (N:C) ratio is 4:1. The large, round nucleus contains from zero to two nucleoli, is usually dark appearing, and has a fi ne chromatin pattern. the cytoplasm stains a distinctive blue (basophilic) color with Wright stain and lacks granules. The distinctive blue color reflects the RNA activity needed to produce the protein required for hemoglobin synthesis. Studies with radioactive iron have demonstrated that most of the iron destined for hemoglobin synthesis is taken into the cell at this stage (Mary, 2001).

1.2.4.2.2. Prorubricyte (Basophilic Normoblast):

The second stage, the prorubricyte or basophilic normoblast, has an overall cell diameter of 12 to 17 mm and is only slightly smaller than the rubriblast. The N:C ratio remains high (4:1); however, this stage demonstrates morphological evidence of increasing maturity. The nuclear chromatin becomes more clumped. Nucleoli are usually no longer apparent. The cytoplasm continues to appear basophilic with a Wright stain. This cell contains no evidence of the pink color that indicates hemoglobin development (Mary, 2001).

1.2.4.2.3. Rubricyte (Polychromatic Normoblast):

Hemoglobin appears for the first time in the third maturational stage, the rubricyte or polychromatic normoblast. At this stage, the overall cell size of 11 to 15 mm is slightly decreased from that of the prorubricyte stage. Further maturation is also demonstrated by the decreased N:C ratio of 1:1. The chromatin continues to become increasingly clumped. The cytoplasm of cells in this stage

shows variable amounts of pink coloration mixed with basophilia; this can give the cell a muddy, light gray appearance (Mary, 2001).

1.2.4.2.4. Metarubicyte (Orthochromic Normoblast);

The rubricyte matures into the metarubicyte or orthochromic normoblast. The overall cell is smaller (8 to 12 mm). The chromatin pattern is tightly condensed in this maturational stage and can be described as pyknotic (dense or compact). In the later period of this stage, the nucleus will be extruded from the cell. The metarubicyte is characterized by an acidophilic (reddish pink) cytoplasm. This coloration indicates the presence of large quantities of hemoglobin. Three mitoses are believed to occur in the 2- to 3-day interval between the rubriblast and the end of the metarubicyte stage. Two thirds of these mitoses have been shown to occur in the rubricyte stage. After this stage, the cell is no longer able to undergo mitosis (Mary, 2001).
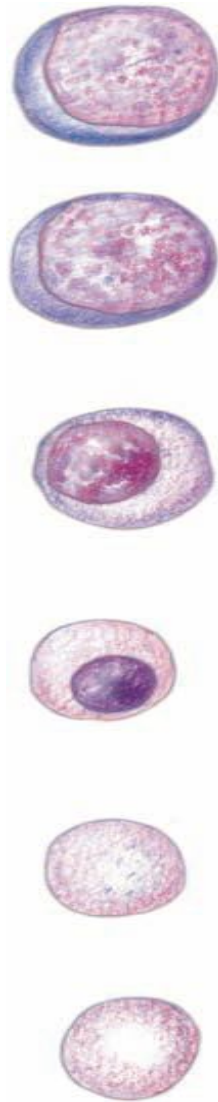
1.2.4.2.5. Reticulocyte:

The reticulocyte stage is the next maturational stage. Part of this phase occurs in the bone marrow, and the later part of the stage takes place in the circulating blood. This cell demonstrates a characteristic reticular appearance caused by remaining RNA if stained with a supravital stain, such as new methylene blue. In a Wright-stained blood smear, young reticulocytes with a high amount of RNA residual have a blue appearance, which is referred to as polychromatophilia. The overall cellular diameter ranges from 7 to 10 mm. This cell is anuclear (Mary, 2001).

1.2.4.2.6. Mature Erythrocyte:

After the reticulocyte stage, the mature erythrocyte is formed. This cell has an average diameter of 6to 8mm. The survivability of erythrocytes can be

determined by using radioactive chromium (51Cr). A shortened life span can be observed in the hemolytic anemias (Mary, 2001).

Pronormoblast (Rubriblast).

Basophilic Normoblast (Prorubricyte).

Polychromatophilic Normoblast (Rubricyte).

Orthochromic Normoblast (Metarubricyte).

Polychromatophilic Erythrocyte (Reticulocyte).

Mature Red Blood Cell (Mature Erythrocyte).   "Respectively".

(Figure 1.2.) Show: Erythrocyte morphology. The morphological development of the erythrocyte is typical of blood cell maturation. The unique difference is that the erythrocyte loses its nucleus (Mary, 2001).
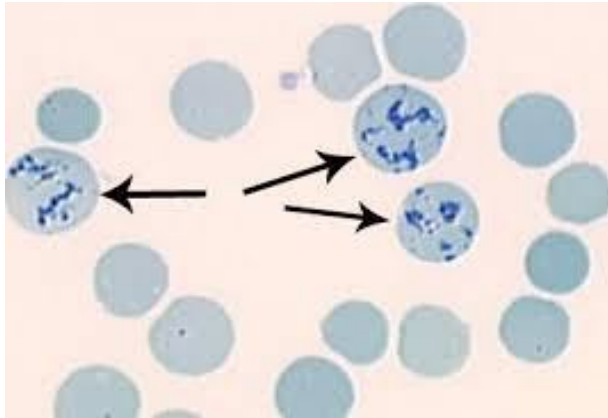
Reticulocytes:

These are the immediate precursors of mature erythrocytes. They are rounded anucleate cells that are about 20% larger in volume than mature red blood cells and appear faintly polychromatic when stained by a Romanowsky method. When stained with a supravital stain such as new methylene blue or brilliant cresyl blue, the diffuse basophilic material responsible for the polychromasia (i.e. ribosomal RNA) appears as a basophilic reticulum. Electron-microscope studies have shown that reticulocytes are rounded cells with a tortuous surface and that in addition to ribosomes they contain mitochondria and autophagic vacuoles. Circulating reticulocytes mature into red cells over a period of 1–2 days during which there is progressive degradation of ribosomes and mitochondria and the acquisition of a biconcave shape (Anna, et. al, 2011).

Reticulocytes actively synthesize hemoglobin and nonhemoglobin proteins. They contain enzymes of the Embden–Meyerhof pathway and the pentose phosphate shunt and, unlike the mature red cells, can also derive energy aerobically via the Krebs cycle that operates in the mitochondria and oxidizes pyruvate to CO_2 and water. Supravitally stained preparations were traditionally used and are still frequently used to assess reticulocyte numbers by microscopy with an eyepiece micrometer disc to facilitate counting (Anna, et. al, 2011).

In normal adults, the reference range for reticulocytes counted in this way is widely accepted to be 0.5–2.0% of the total circulating erythrocyte plus reticulocyte population. The usefulness of the reticulocyte percentage is increased by applying a correction for the hematocrit and the corrected reticulocyte percentage (usually corrected to a hematocrit of 0.45) is obtained by multiplying

the observed percentage by [patient's hematocrit $\div$ 0.45]. Although several laboratories still express reticulocyte counts as a percentage, the absolute reticulocyte count (i.e. the total number per liter of blood) is clinically more useful. The latter is directly proportional both to the rate of effective erythropoiesis and to the average maturation time of blood reticulocytes. In normal adults the absolute reticulocyte count determined by microscopy is 20–110×10^9/l. Reticulocytes can be counted using automated machines employing flowcytometry and laser light after staining their RNA with fluorescent reagents such as acridine orange, thioflavin T, thiazol orange or auramine O. There are also automated methods in which the RNA is stained with supravital basic dyes and the extent of staining quantified using light absorbance or scatter. Results obtained by these automated methods are more reproducible than when counted by the traditional manual method as much larger numbers of eticulocytes are counted. The accepted reference range for reticulocytes in adults when counted by automated fluorescent-based methods is 20–120×10^9/l. The absolute reticulocyte count has also been shown to be higher in men than women. Reference values do depend on the method of measurement used and each laboratory should determine its own reference range. Semi-automated and fully automated discrete reticulocyte counters and some fully automated multiparameter hematology analyzers also provide various reticulocyte maturation parameters based primarily on the intensity of fluorescence (i.e. the amount of RNA), or, in the case of cells stained supravitally with a basic dye, on the extent of absorbance or scatter. These parameters include the immature reticulocyte fraction (immature reticulocytes have more RNA than mature ones), mean reticulocyte hemoglobin content and concentration and mean reticulocyte volume. Although these parameters have been shown to be of value in the

assessment of certain clinical situations, they are not in regular use in clinical practice (Anna, et. al, 2011).

(Figure 1.3.) Show: Microscopical picture of supravital staining of red cells to show reticulocytes (Mary, 2001).

1.2.5. Pregnancy: pregnancy is the most important physiological state for human kind since it assures continuation of the species. Pregnancy produces major physical alterations in the mother support the fetus as it develops the capability of independent existence and introduces a new organ in the form of the placenta that provides the link between the two (Hytten, 1985).

1.2.5.1. Physiological changes during pregnancy: maternal physiological changes during pregnancy are the normal adaptations that a woman undergoes during pregnancy to better accommodate the embryo or fetus. (Milman, et al, 2000).

1.2.5.1.1. Physical changes: one of the most noticeable alterations in pregnancy is the gain of weight, the enlarging uterus, the growing fetus in the placenta and the acquisition of fat and water retention; all contribute to this increase in weight.

The weight gain varies from person to person and can be any where from 5 pounds (2.3 kg) to over 100 pounds (45 kg) (Koller, et al, 1979).

1.2.5.1.2. Hormonal changes: pregnant woman experience adjustment in their endocrine system. Levels of progesterone and estrogen rise continually throughout pregnancy, suppressing the hypothalamic axis and subsequently the menstrual cycle estrogen mainly produced by placenta and associated with fetal wellbeing. Women also experience increased Human Chorionic Gonadotropin (HCG); which is produced by the placenta. Prolactin levels increase due to maternal pituitary gland enlargement by 50%. This mediate changes in the structure of the mammary gland from the ductal to lobulo-alveolar. Parathyroid hormone is increased which leads to increases of calcium uptake in the gut and reabsorption by the kidneys. Adrenal hormones such as cortisol and aldosterone also increased, human placental Lactogen (HPL) is produced by the placenta and stimulates lypolysis and fatty acids metabolism by the woman, conserving blood glucose for use by the fetus. It can also decrease maternal tissue sensitivity to insulin; resulting in gestational diabetes (Koller, et al, 1979).

1.2.5.1.3. Musculoskeletal changes: the body's posture changes as the pregnancy progresses; the pelvis tilts and the back arches to help keep balance, poor posture occurs naturally from the stretching of the woman's abdominal muscles as the fetus growth. These muscles are less able to contract and keep the lower back in proper alignment. The pregnant woman has a different pattern of gait. The step lengthens as the pregnancy progresses due to weight gain and change in posture. On average, a woman's foot can grow by half size or more during pregnancy (Koller, et al, 1979).

1.2.5.1.4. Cardiovascular changes: the woman is the sole provider of nourishment for the embryo and later; the fetus; and also her plasma and blood volume slowly increased by 40- 50% over the course of the course of the pregnancy to accommodate the changes. The increase is mainly due to an increase in plasma volume through increased aldosterone. It results in an increase in heart rate (15 peats/ minute more than normal), stroke volume and cardiac output. The systemic vascular resistance also drops due to the smooth muscle relaxation and overall vasodilatation caused by elevated progesterone leading to a fall in blood pressure (Koller, et al, 1979).

1.2.5.1.5. Metabolic changes: during pregnancy, both protein metabolism and carbohydrate metabolism are affected. One kilogram of extra protein is deposited with half going to fetus and placenta and another half going to uterine contractile proteins, breast glandular tissue, plasma protein and haemoglobin. Increased requirement for nutrients is given by fetal growth and fat deposition. Changes are caused by steroid hormones, lactogen and cortisol. Increased liver metabolism is also seen; with increase maternal glucose levels (Koller, et al, 1979).

1.2.5.1.6. Renal changes: a pregnant woman may experience an increase in kidneys and ureter size. The glomerular Filtration Rate (GFR) commonly increases by 50%, returning to normal around 20weeks postpartum. There is decreased Blood Urea Nitrogen (BUN) and creatinine and glucosurea (due to saturated tubular reabsorption) may be seen (Koller, et al, 1979).

1.2.5.1.7. Gastrointestinal changes: during pregnancy, woman can experience nausea and vomiting (morning sickness); which may be due to elevated HCG and should resolve by 14 to 16 weeks. Additionally, there is prolonged gastric empty time, decreased gastroesophagal sphincter tone, which can lead to acid reflux and

decreased colonic motility; which leads to increased water absorption and constipation (Koller, et al, 1979).

1.2.5.1.8. Normal Haematological changes: the too most pronounced hematological changes during pregnancy involve increases in plasma volume and total red cells mass. The plasma volume rises by 30% while the total red cell mass and number of red cells increases by only about 20%. The result is fall in hematocrit. This decline in hematocrit is called the "physiological anemia" or "dilutional anemia" of pregnancy. True anemia represents a fall in oxygen transport capacity of blood relative to the normal physiological state. This is not the case during pregnancy where the oxygen-carrying capacity is higher than the non-pregnant state. (Riikonen, et al, 1994). The rise in plasma volume begins at about 6weeks into the pregnancy. The rise is initially rapid but pace slows after about 30. The plasma volume at term is about 1200ml greater than in non-pregnant baseline, which translates into an increase of nearly 50%. The red cell mass also increases over this time, with a net rise that by term ranges between 250 and 400ml. the hematocrit normally declines into second trimester but rises slowly thereafter. The most equitable means of approaching the problem is to assign 11g/dl as the lower limit of normal hemoglobin values during pregnancy. Interestingly, high hemoglobin values during pregnancy are not felicitous findings. Unexplained values above 13g/dl are associated with poor fetal outcome, including intrauterine growth retardation, low birth weight and preterm birth. Not surprisingly, a rise in serum erythropoietin values appears to be a key factor in red cell mass expansion during pregnancy (Koller, et al, 1979).

1.2.6. Miscarriage: The medical term for a miscarriage is spontaneous abortion, but the condition is not an abortion in the common definition of the term. According to the March of Dimes, as many as 50% of all pregnancies end in miscarriage -- most often before a woman misses a menstrual period or even knows she is pregnant. About 15% of recognized pregnancies will end in a miscarriage. More than 80% of miscarriages occur within the first three months of pregnancy. Miscarriages are less likely to occur after 20 weeks gestation; these are termed late miscarriages (webMD, 2005).

1.2.6.1. Symptoms of a Miscarriage: Symptoms of a miscarriage include:

Bleeding which progresses from light to heavy.

Severe cramps.

Abdominal pain.

Fever.

Weakness.

Back pain (webMD, 2005).

1.2.6.2. Causes Miscarriage:

The causes of miscarriage are not well understood. Most miscarriages that occur in the first trimester are caused by chromosomal abnormalities in the baby. Chromosomes are tiny structures inside the cells of the body which carry many genes. Genes determine all of a person's physical attributes, such as sex, hair and eye color, and blood type. Most chromosomal problems occur by chance and are not related to the mother's or father's health (webMD, 2005).

Miscarriages are also caused by a variety of other factors, including:

- Infection.

- Exposure to environmental and workplace hazards such as high levels of radiation or toxic agents.

- Hormonal problems.

- Uterine abnormalities.

- Incompetent cervix (the cervix begins to widen and open too early, in the middle of pregnancy, without signs of pain or labor).

- Lifestyle factors such as smoking, drinking alcohol, or using illegal drugs.

- Disorders of the immune system, including lupus.

- Severe kidney disease.

- Congenital heart disease.

- Diabetes that is not controlled.

- Thyroid disease.

- Severe malnutrition.

In addition, women may be at increased risk for miscarriage as they get older. Studies show that the risk of miscarriage is 12% to 15% for women in their 20s and rises to about 25% for women at age 40. The increased incidence of chromosomal abnormalities contributes to the age-related risk of miscarriage.

There is no proof that stress or physical or sexual activity causes miscarriage. (webMD, 2005).

1.3. Rationale

This project was performed due to importance of pregnant women's & their baby's health, & due to importance to control the physiological anemia of pregnancy that caused by plasma volume increasing.

Based on previous studies that demonstrated larger immature erythrocytes during pregnancy; I want to show the effect of pregnancy and miscarriage on reticulocytes count and erythropoiesis among pregnant women.

1.4. Objectives

1.4.1. General objective:

➢ To investigate the effects of miscarriage, number of pregnancies and stage of pregnancy on reticulocyte count and Reticulocyte Production Index (RPI) in pregnant women.

1.4.2. Specific objectives:

➢ To evaluate Reticulocyte count during pregnancy.
➢ To evaluate RPI during pregnancy.
➢ To investigate the changes in erythropoiesis during pregnancy.
➢ To evaluate the production and releasing of red blood cells by bone marrow.
➢ To evaluate the affection of miscarriage on erythropoiesis during gestation.

Chapter Two

Materials and Methods

2.1. Study Design:

This is a descriptive cross sectional study, performed at White Nile State in Kosti Teaching Hospital- Obstetric part; during a period from March 2014- June 2014. This study include Sudanese pregnant women.

2.2. Inclusion criteria:

Only pregnant women were included in this study.

2.3. Exclusion criteria:

Young girls were excluded from this study.

2.4. Sampling:

Randomized system was used to collect 100 blood study subject and 30 samples as control after verbal consent taken from each participant.

2.5. Sample collection:

2.5ml whole blood was drawn from an antecubital vein by means of diposable plastic syringe into EDTA anticoagulant treated containers.

The skin was cleaned with 70% alcohol and allowed to dry before being punctured.

After detaching the needle the blood was delivered carefully from syringe into container.

2.6. Ethical consideration:

All participants were informed about this study and its benefits on their health.

2.7. Data analysis and presentation:

The data was analyzed by using SPSS test (t-independent test)

2.8. Method:

2.8.1.Hematocrit and Red Blood Cells count:

For each sample of EDTA Blood the following hematimetric variables: Red Blood Cells (RBC), hematocrit (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Absolute Neutrophils Count (ANC) and Absolute Lymphocytes Count (ALC); were determined in an automated counter (Sysmex KX-21N Model-Japan).

2.8.1.1. Manual method:

The conventional method for determination of hemogram is by using certain solutions reagents and diluents fluids.

For RBC's: was diluted using 1%formal citrate (Tri Sodum Citrate and formalin) which allowing count manually in hemocytometer (champer) under microscope x40 lens.

For hematocrit: by using capillary tubes and special centrifuge.

2.8.1.2. Automated method:

Principally sysmex analyzer is based on the electronic resistance (impedance) detection method for counting and sizing recognition of the leukocytes, erythrocytes and thrombocytes; using three hydraulic systems for WBC, RBC, Platelets and Hemoglobin; the results was displayed on the liquid crystal displayer (LCD) with histogram and printed out in thermal paper.

Reagents and materials: commercial close system reagents were provided by sysmex 21 operators and consist of:

- Cell bag and stromatolyser.
- Detergent and cell cleaner.

Principle of hematological analyser:

Measurement of blood cells (RBC's, WBC's & Platelets) and hemoglobin concentration obtained by aspiration of small volume of well mixed K_3EDTA blood by sample probe and mixed with isotonic diluents in nebulizer. Diluted mixture aspiration was delivered to RBC aperture bath for providing information about RBC and Platelet based on cell sizes, particles of 2 to 20 fl counted as platelets; above 36fl counted as red cells. Some portion of aspirated mixture induced into WBC bath in which hemolytic reagent (stromatolyser) was added automatically to measure hemoglobin concentration in build calorimeter, based on cyanomethemoglobin method (HiCN). Blood cells counted and size information generated in triplicate pulses according to electronic conductivity, and translated into digital number using in build calculater programmed and designed for that RBC,WBC counts, hence three values were directly measured (RBC, WBC & Hb), and displayed on LCD. Other values of red cell indices,

platelet counts, leucocytes differential and absolute count calculated from given information and automated constructed histograms, the results printed out according to the setting mode.

2.8.2. Reticulocytes count:

Reticulocyte count measures the percentage of reticulocytes (slightly immature red blood cells) in the blood.

The test is done to determine if red blood cells are being created in the bone marrow at an appropriate rate. The number of reticulocytes in the blood is a sign of how quickly they are being produced and released by the bone marrow (Rockville, et al., 2014)

2.8.1.1. Reagents:

(1) New Methylene Blue Solution was prepared by dissolving 0.5 grams of new methylene blue, 1.4 grams of potassium oxalate, and 0.8 grams of sodium chloride in distilled water. Dilute to 100 ml. was filtered before use.

(2) Brilliant Cresyl Blue Solution was prepared by dissolving 1.0 grams of brilliant cresyl blue in 99 ml of .85 per cent sodium chloride. Was filtered before use (David and Heiserman, 2004).

2.8.1.2. Principle:

Non-nucleated immature erythrocytes contain nuclear remnants of RNA and the cell is known as a reticulocyte. To detect the presence of this RNA, the red cells must be stained while they are still living. This process is called supravital

staining. With supravital staining, the RNA appears as a reticulum within the red cell (David and Heiserman, 2004).

2.8.1.3. Requirements:

1. EDTA Blood sample.

2. Reticulocytes stain.

3. Test tubes.

4. Water bath.

5. Slides.

6. Microscope.

2.8.1.4. Procedure:

New methylene blue was mixed with equal volume of venous whole blood in a small test tube. And then allowed to stand for a minimum of 15 minutes; his allows the reticulocytes adequate time to take up the stain. At the end of 15 minutes, the contents of the tube were mixed well. A small drop of the mixture was placed on a clean glass slide a thin smear was prepared. The slide was placed on the microscope stage and by using the low power objective the thin portion of the smear in which the red cells are evenly distributed and are not touching each other was located. Oil immersion magnification was switched and counts the number of reticulocytes in 5 fields of 200 RBCs (David and Heiserman, 2004).

2.8.1.5. Calculation:

Reticulocyte % = (No of reticulocytes/ 1000 red blood cells observed) *100.

Corrected Reticulocytes Count:

CRC= Reticulocytes% * patient's PCV/ normal PCV.

Absolute Reticulocyte Count:

ARC= REticulocytes% * RBC's count/ 100.

Reticulocyte Production Index:

RPI= CRC/ maturation time in peripheral blood.

Hct (L/L)	Maturation Time/ Days	
0.40- 0.45	1	
0.35- 0.39	1.5	
0.25- 0.34	2	
0.15- 0.24	2.5	
<0.15	3	(David L. Heiserman; 2004).

2.8.3. Normal Values:

- Retics%:

(1) At birth: 1.5 to 6.0 percent, but falls to adult range by the end of second week of life.

(2) Adults (both sexes): 0.5 to 1.5 percent (David and Heiserman, 2004).

- RBC's count: 4.0- 5.0 * 10^6/mm^3
- Hct: 36- 48% (Bruce, 2005).

Chapter three

Results

Descriptives

Table "1" show findings of abortion, pregnancy and control.

Variable	Abortion (mean ± SD)	Pregnancy (mean ± SD)	Control (mean ± SD)
Reticulocyte count	2.1± 0.6	1.3± 0.3	0.4 ±0.3
RBC's count	4.0 ±0.5	4.3± 0.5	3.1 ±0.9
Hematocrit%	34.6± 3.0	35.9 ±3.0	27.4 ±8.2
CRC	1.7± 0.4	1.0 ±0.3	0.2± 0.06
ARC	0.07 ±0.04	0.04± 0.03	0.02
RPI	1.2 ±0.5	0.8 ±0.3	0.3± 0.2

Tables "2" and "3" and figures 3.1. and 3.2. show comparisons between case and control on reticulocyte production.

T-Test:

Group Statistics

	Sample	N	Mean	Std. Deviation
RETICS COUNT %	pregnant	100	1.496	.5447
	control	30	.487	.3441
RPI	pregnant	100	.882	.4159
	control	30	.301	.4222

Independent Samples Test

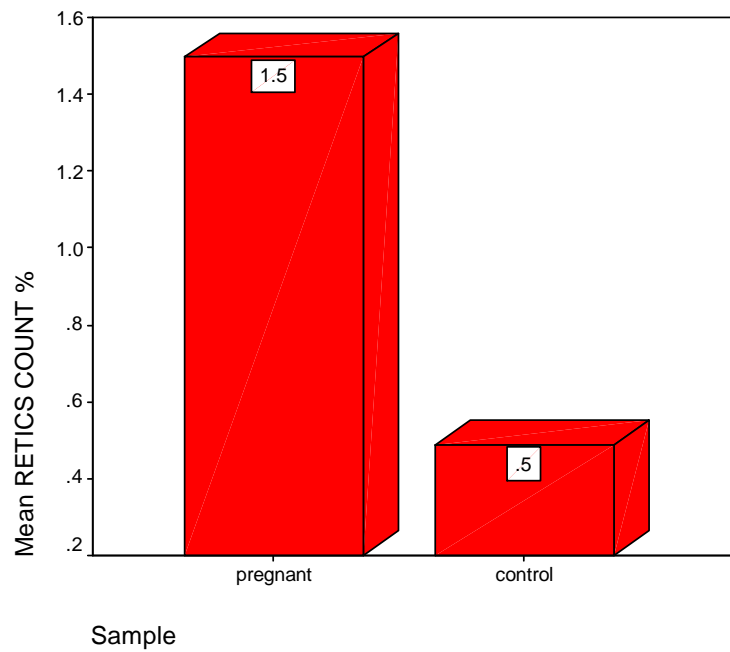|	t-test for Equality of Means			
			95% Confidence Interval of the Difference	
	df	Sig. (2-tailed)	Lower	Upper
RETICS COUNT %	128	.000	.8008	1.2179
RPI	128	.000	.4094	.7532

Fig3.1. Comparison of reticulocytes count between cases and control.

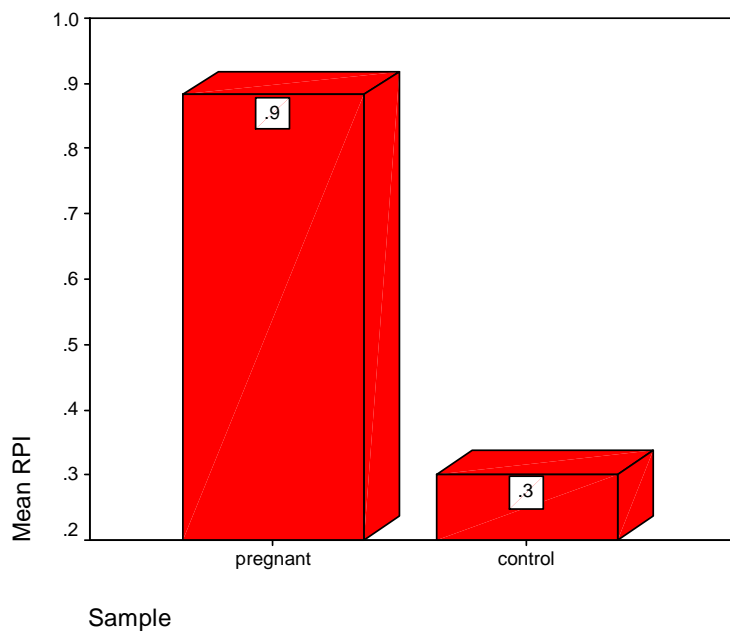**Fig3.2. Comparison of RPI between cases and control.**

Table "4" and "5" and figures 3.3 and 3.4. show effect of age on reticulocyte production.

T-Test:

Group Statistics

	AGE GROUP/ YEAR	N	Mean	Std. Deviation
RETICS COUNT %	25 AND LESS	59	1.473	.4748
	26 AND MORE	41	1.529	.6369
RPI	25 AND LESS	59	.859	.3593
	26 AND MORE	41	.916	.4889

Independent Samples Test

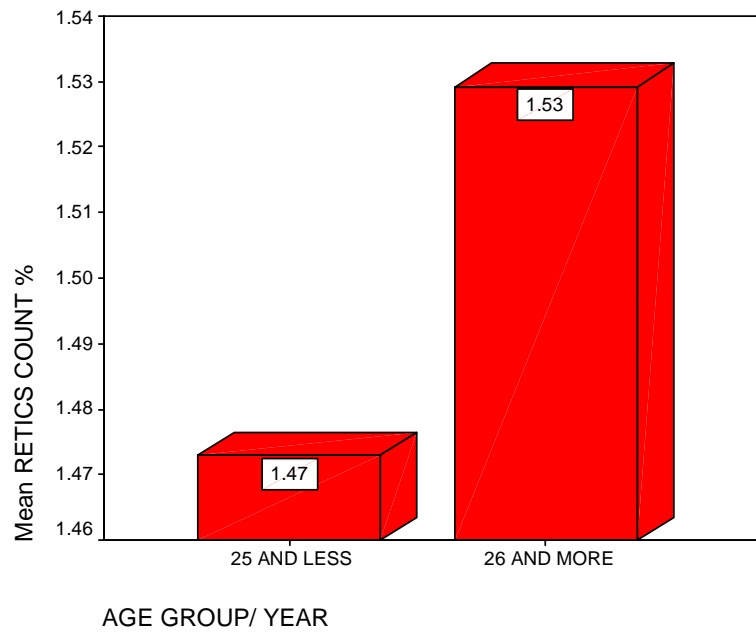|	t-test for Equality of Means			
	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
RETICS COUNT %	98	.613	-.056	.1112
RPI	98	.509	-.056	.0848

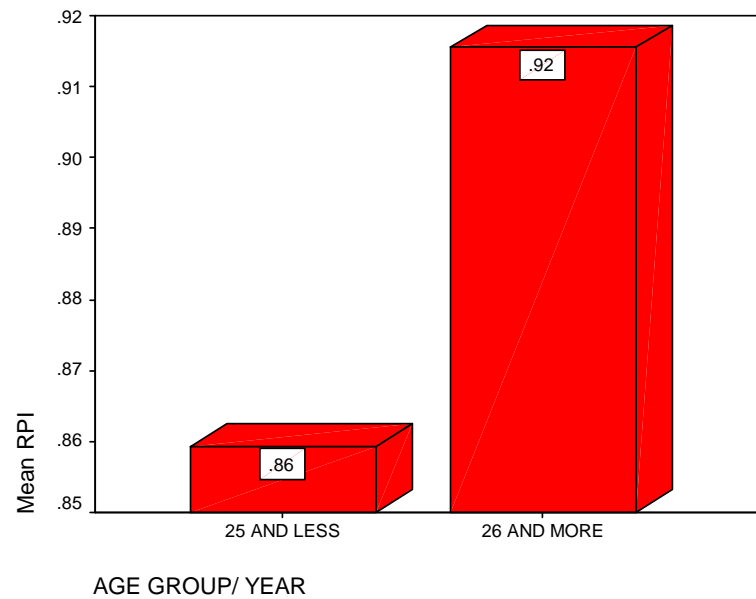**Fig3.3. Comparison of reticulocytes count between age groups.**

Fig3.4. Comparison of RPI between age groups.

Tables "6" and "7" and figures 3.5 and 3.6 show effect of numbers of pregnancies on reticulocyte production.

T-Test:

Group Statistics

	NO. OF PREGNANCIES	N	Mean	Std. Deviation
RETICS COUNT %	6 AND LESS	92	1.479	.5340
	7 AND MORE	8	1.687	.6664
RPI	6 AND LESS	92	.881	.4261
	7 AND MORE	8	.900	.2927

Independent Samples Test

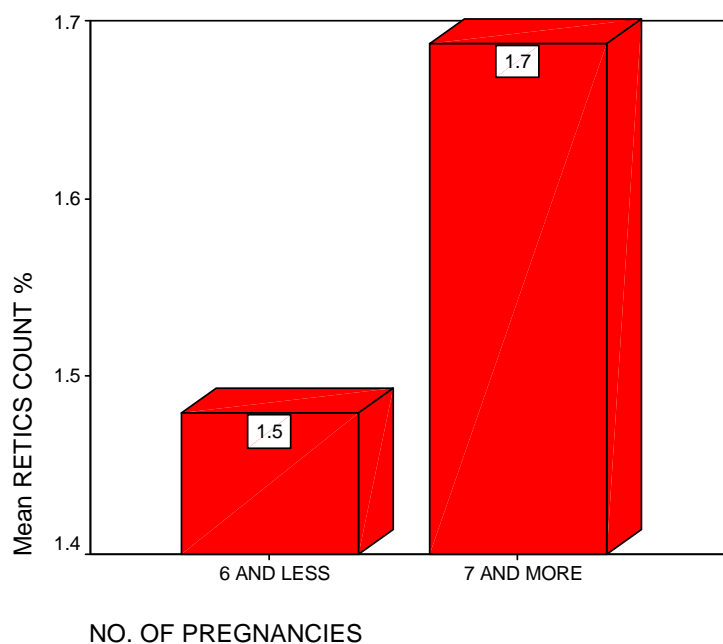|	t-test for Equality of Means			
	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
RETICS COUNT %	98	.302	-.208	.2007
RPI	98	.903	-.019	.1541

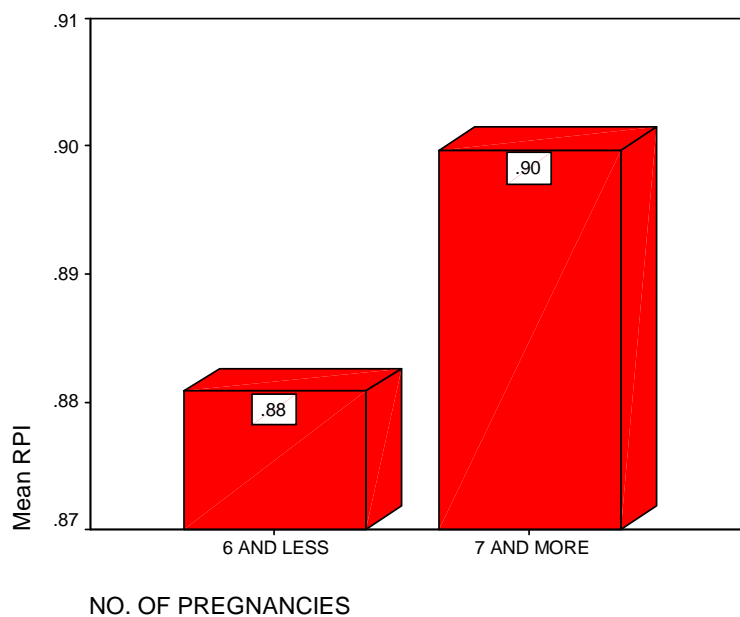**Fig3.5. Comparison of reticulocytes count between No. of pregnancy.**

Fig3.6. Comparison of RPI between No. of pregnancy.

Tables "8" and "9" and figures 3.7. and 3.8. show effect of abortion on reticulocyte production.

T-Test:

Group Statistics

	PREVIOS ABORTION	N	Mean	Std. Deviation
RETICS COUNT %	yes	25	2.124	.5681
	no	75	1.287	.3387
RPI	yes	25	1.197	.5097
	no	75	.777	.3201

Independent Samples Test

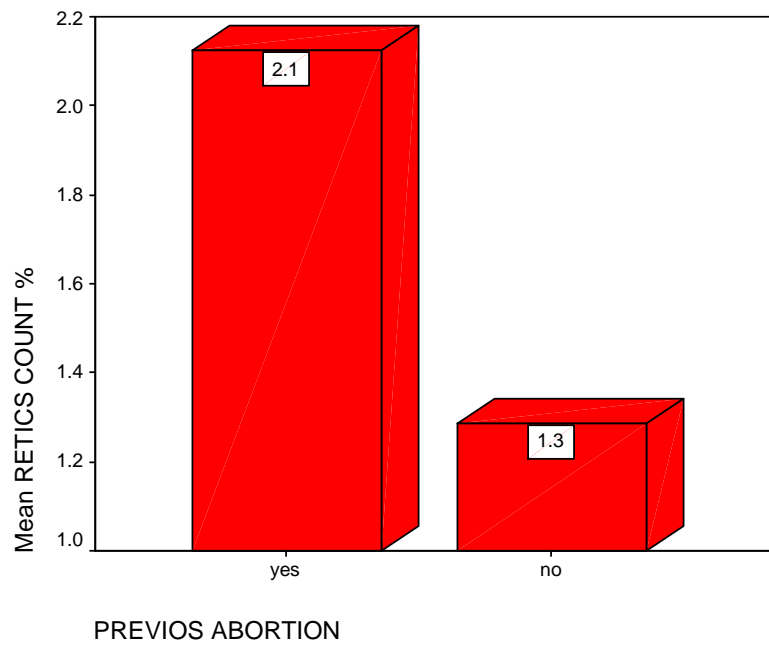|	t-test for Equality of Means			
	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
RETICS COUNT %	98	.000	.837	.0940
RPI	98	.000	.420	.0867

Fig3.7. Comparison of reticulocytes count between miscarriage frequencies.
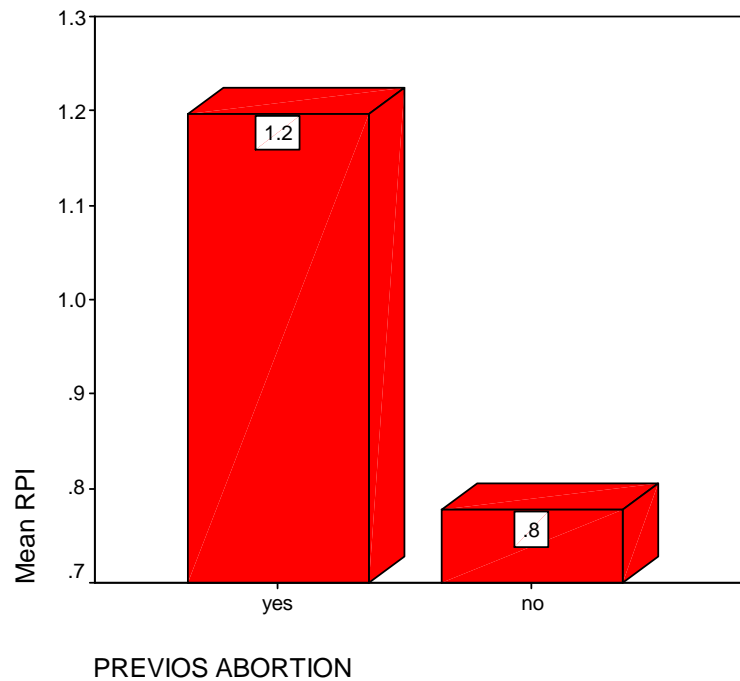

Fig3.8. Comparison of RPI between miscarriage frequencies.

Chapter four

Discussion, Conclusion and Recommendations

4.1. Discussion

The study aimed to evaluate the effect of pregnancy and miscarriage on reticulocyte count and RPI; then compared with non pregnant females as control group.

The study showed the mean of reticulocyte count through pregnant women with previous abortion (2.1) reflect significant variation results when compared with women with no previous abortion (1.3) P.value (0.00).

The mean of reticulocyte count in pregnant women (1.5) is elevated when compared with control group mean.This result is agreed with previous result of Choi JW, Pai SH; change in erythropoiesis with gestational age during pregnancy. Department of Clinical Pathology, Inha University Hospital, Jung-gu, Inchon, Korea , jan2001.

And mean of retics through age group less than 25 years (1.5) showed no statistical differences when compared with 26years and above (1.5) P.value (0.6).

Mean of pregnancies 6times and less (1.5) showed no variation when compared with 7pragnancies and more (1.7) P.value (0.3).

RPI mean through pregnant women with previous abortion (1.2) showed significant variation results when compared with women with no previous abortion (0.8) P.value (0.00).

Mean of RPI in pregnant women (0.9) showed significant variation result when compared with control group mean (0.3) P.value (0.00).

And mean of RPI through age group less than 25 years (0.9) showed no statistical differences when compared with 26years and above (0.9) P.value (0.5).

Mean of RPI among pregnancies 6times and less (0.9) showed no variation when compared with 7pragnancies and more (0.9) P.value (0.9).

4.2. Conclusion

The result of this study showed that the production and releasing of reticulocytes by bone marrow during pregnancy is increased when compared with non pregnant women (control group), and erythropoiesis is enhanced with pregnancy with previous abortion when compared with pregnant women without abortion that reflected by retics count and RPI.

4.3. Recommendations

*Marked elevation of reticulocyte count and RPI with abortion reflects the danger of abortion and importance of antenatal care of women.

*Use of other more advanced techniques to count retics such as flowcytometer and laser light.

* It's recommended to carry out this study among the three trimesters and compare between them.

* Researches must be continued and in all heamatological parameters to be more familiar with heamatological changes during pregnancy.

* Raising the awareness of married women and especially pregnant women and who had history of abortion by health education and importance of periodic antenatal check up through various forms such as educational institutes, non-governmental organizations and community leaders.

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Appendix (1)

Sudan University of Sciences & Technology

Faculty of Medical Laboratories- Haematology department

Questionnaire about

Pregnant women at third trimester

Sample No (   )

Patient's inf:

Name: …………………..………………………………………

Age: ……………………………………………Years.

Address: …………………………………………………..

Clinical remark :

No. of pregnancy: ………………………

Stage of pregnancy: ……………………….. month.

Abortion: Yes [ ]   No [ ]

Lab. Investigation:

-Reticulocytes count= ………………%   RBC's= …………..*10^6/cumm

-RPI= …………………..   PCV= …………..%

Signature: ..nihad bakry**..**   Date: …………………

2014

Appendix (2)

Proerythroblast	**Basophilic Normoblast**	**Polychromatic Normoblast**
Orthochromatic Normoblast	**Reticulocyte**	**Erythrocyte**
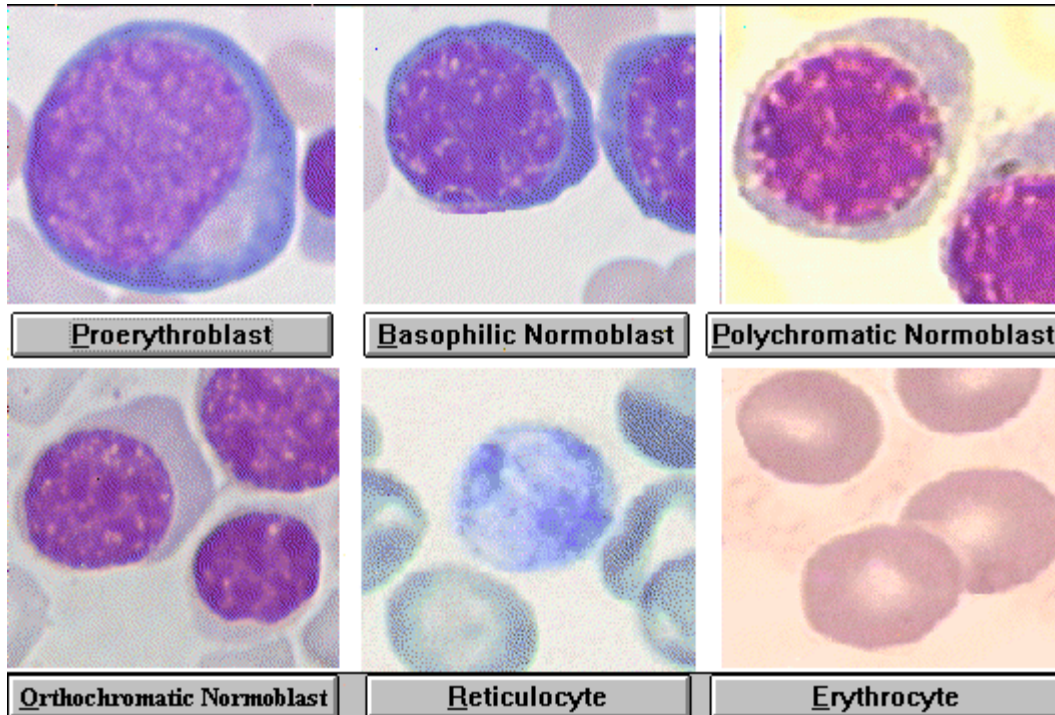
Appendix (3)

Whole blood sample collected in EDTA treated container.