1.0: Introduction and Literature Review

1.1: General Introduction:

Reference range for a population can be established from measurements on relatively small number of subjects if they are assumed to be representative of the population as whole. [3]

Blood is a bodily fluid that delivers necessary substances such as nutrients , it is composed of blood cells suspended in plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume) and contains dissipated proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Blood performs many important functions within the body such as supplying the tissues with oxygen , supplying of nutrients such as glucose, amino acids, and fatty acids, Removal of waste such as carbon dioxide, urea, and lactic acid, regulation of body pH and temperature, coagulation and immunity.The blood cells are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in blood are red blood cells carry oxygen to the cells and transports metabolic waste products away from those same cells.[8]

White blood cells have fundamental roles in defense against invading micro-organisms and the recognition and destruction of neoplastic cells as well as their role in acute inflammatory reactions furthermore, through their phagocytic functions; white blood cells are influential in clearing senescent and apoptotic cells, hence allowing tissue repair and remodeling. Production of various cytokines by white blood cells influences the functions of other cells and affects processes such as cellular and humoral immunity, and allergic phenomena. The phagocytic actions of white blood cells can cause damage to the host tissue, leading

to inflammation. This occurs either as a by-product of their microbial killing actions or as a direct attack on the host in autoimmune disorders.(3) Hematopoiesis is the process of blood cell development, follows a definite sequence of sites from embryonic life to fetal life to childhood to adult life. In abnormal situations, blood production may revert to a more primitive state, referred to as extramedullary hematopoiesis. The stem cell is the first in a sequence of steps of hematopoietic cell generation and maturation. Hematopoietic cells can be divided into three phases according to cell maturity. The multipotential stem cell is the progenitor of the two major cell lines: lymphoid and nonlymphoid. Colony-forming units precede the blast stage of cell development. Hematopoietic growth factors regulate the proliferation and differentiation of progenitor cells and the function of mature blood cells. These factors are being used to treat a variety of diseases and disorders. Each cellular element has a name and associated characteristics for each stage of development. Certain maturational characteristics are shared by most hematopoietic cells. Characteristics such as overall size and N:C ratio are important in determining the stages of development. Nuclear characteristics, such as the presence of nucleoli and chromatin patterns, vary with cell type and cell maturity. Cytoplasmic features, such as color and the presence of granules, must be carefully observed in a peripheral blood examination. The presence of granules is indicative of the presence of specific cell types and is a feature of cellular age. (4)

Leukemia is caused by the mutation of the bone marrow pluripotent or most primitive stem cells. This neoplastic expansion results in abnormal, leukemic cells and impaired production of normal red blood cells, neutrophils, and platelets. As the mutant cell line takes hold and normal hematopoiesis is inhibited, the leukemic cells spill into the peripheral blood and invade the reticuloendothelial tissue, specifically the spleen,

liver, lymph nodes, and, at times, central nervous system. The leukemic stem cells have atypical growth and maturation capability. The mutant clone may demonstrate unique morphologic, cytogenetic, and immunophenotypic features that can be used to aid in the classification of the particular type of leukemia. Many of the leukemias have similar clinical features, but regardless of the subtype, the disease is fatal if left untreated. **(6)**

1.2: Literature Review:

1.2.1: Blood:

Blood is a bodily fluid that delivers necessary substances such as nutrients; it is composed of blood cells suspended in blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume) and contains dissipated proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood. The blood cells are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in blood are red blood cells carry oxygen to the cells and transports metabolic waste products away from those same cells. Medical terms related to blood often begin with hemo- or hemato- (also spelled haemo- and haemato-) from the Greek word (haima) for "blood". In terms of anatomy and histology, blood is considered a specialized form of connective tissue, given its origin in the bones and the presence of potential molecular fibers in the form of fibrinogen. **(8)**

1.2.1.1: Constituents of human blood:

Blood accounts for 7% of the human body weight, with an average density of approximately 1060 kg/m3, very close to pure water's density of 1000 kg/m3. The average adult has a blood volume of roughly 5 -6 liters, which is composed of plasma and several kinds of cells. These blood cells (which are also called corpuscles or "formed elements") consist of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7% cells:

One micro liter of blood contains:

1.2.1.1.1: 4.7 to 6.1 million (male), 4.2 to 5.4 million (female) erythrocytes: Red blood cells contain the blood's hemoglobin and distribute oxygen. Mature red blood cells lack a nucleus and organelles in mammals. The red blood cells (together with endothelial vessel cells and other cells) are also marked by glycoproteins that define the different blood types. The proportion of blood occupied by red blood cells is referred to as the hematocrit, and is normally about 45%.**(8)**

1.2.1.1.2: 4,000–11,000 leukocytes: White blood cells are part of the body's immune system; they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances. The cancer of leukocytes is called leukemia.

1.2.1.1.3: 200,000–500,000 thrombocytes: Also called platelets, thrombocytes are responsible for blood clotting (coagulation). They change fibrinogen into fibrin. This fibrin creates a mesh onto which red blood cells collect and clot, which then stops more blood from leaving the body and also helps to prevent bacteria from entering the body. **(8)**

About 55% of blood is blood plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color. The blood plasma volume totals of 2.7–3.0 liters in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid. **(8)**

1.2.1.1.4: Other important components include: Serum albumin, Blood-clotting factors (to facilitate coagulation), Immunoglobulins (antibodies), lipoprotein particles, other proteins, electrolytes (mainly sodium and chloride)

1.2.1.2: Blood Functions:

- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids).
- Removal of waste such as carbon dioxide, urea, and lactic acid.
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies.
- Coagulation, which is one part of the body's self-repair mechanism (blood clotting after an open wound in order to stop bleeding).
- Messenger functions, including the transport of hormones and the signaling of tissue damage.
- Regulation of body pH.
- Regulation of body temperature.
- Hydraulic functions. **(8)**

1.2.2: Hematopoiesis:

1.2.2.1: Production and degradation of blood cells:

The various cells of blood are made in the bone marrow in a process called hematopoiesis which is (from Ancient Greek "blood" to make") (or hematopoiesis in American English; sometimes also haemopoiesis or hemopoiesis) is the process of blood cell production, differentiation, and development. The hematopoietic system consists of the bone marrow, liver, spleen, lymph nodes, and thymus. All cellular blood components are derived from haematopoietic cells. **(2)**

1.2.2.2: Bone marrow sites and function:

Bone marrow is found within the cavities of all bones and may be present in two forms: yellow marrow, which is normally inactive and composed mostly of fat (adipose) tissue, and red marrow, which is normally active in the production of most types of leukocytes, erythrocytes, and

thrombocytes. The bone marrow is one of the body's largest organs. It represents approximately 3.5% to 6% of total body weight and averages around 1,500 g in adults, with the hematopoietic marrow being organized around the bone vasculature. The bone marrow consists of hematopoietic cells (erythroid, myeloid, lymphoid, and megakaryocyte), fat (adipose) tissue, osteoblasts and osteoclasts, and stroma. Hematopoietic cell colonies are compartmentalized in the cords. Following maturation in the hematopoietic cords, hematopoietic cells cross the walls of the sinuses, specialized vascular spaces, and enter the circulating blood. During the first few years of life, the marrow of all bones is red and cellular. The red bone marrow is initially found in both the appendicular and the axial skeleton .In young people but progressively becomes confined to the axial skeleton and proximal ends of the long bones in adults. By age 18, red marrow is found only in the vertebrae, ribs, sternum, skull bones, pelvis, and to some extent the proximal epiphyses of the femur and humerus. In certain abnormal circumstances, the spleen, liver, and lymph nodes revert back to producing immature blood cells (extramedullary hematopoiesis). In these cases, enlargement of the spleen and liver is frequently noted on physical examination. This situation suggests that undifferentiated primitive blood cells are present in these areas and are able to proliferate if an appropriate stimulus is present. This situation occurs under the following conditions: 1.When the bone marrow becomes dysfunctional in cases such as aplastic anemia, infiltration by malignant cells, or over proliferation of a cell line (e.g., leukemia). When the bone marrow is unable to meet the demands placed on it, as in the hemolytic anemia. (2)

1.2.2.3: Sites of haemopoiesis:

In the first few weeks of gestation the yolk sac is the main site of haemopoiesis. However, definitive haemopoiesis derives from a population of stem cells first observed on the dorsal aorta termed the AGM (aorta-gonads-mesonephros) region. These common precursors of endothelial and haemopoietic cells (haemangioblasts) are believed to seed the liver, spleen and bone marrow and from 6 weeks until 6-7 months of fetal life the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth.The marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses and mature cells are released into the sinusspaces, the marrow microcirculation and so into the general circulation. In infancy all the bone marrow is haemopoietic but during childhood there is progressive fatty replacement of marrow throughout the long bones so that in adult life haemopoietic marrow is confined to the central skeleton and proximal ends of the femurs and humeri. Even in these haemopoietic areas, approximately 50% of the marrow consists of fat. The remaining fatty marrow is capable of reversion to haemopoiesis and in many diseases there is also expansion of haemopoiesis down the long bones. Moreover, the liver and spleen can resume their fetal haemopoietic role. [2]

1.2.2.4: Haematopoietic stem cells (HSCs): Haematopoietic stem cells (HSCs) reside in the medulla of the bone (bone marrow) and have the unique ability to give rise to all of the different mature blood cell types and tissues. HSCs are self-renewing cells: when they proliferate, at least some of their daughter cells remain as HSCs, so the pool of stem cells does not become depleted. The other daughters of HSCs (myeloid and

lymphoid progenitor cells), however can commit to any of the alternative differentiation pathways that lead to the production of one or more specific types of blood cells, but cannot self-renew. This is one of the vital processes in the body. **(7)**

1.2.2.4.1: Types of Human Stem Cells:

1.2.2.4.1.1: Totipotential stem cells: These cells are present in the first few hours after an ovum is fertilized. Totipotential stem cells, the most versatile type of stem cell, can develop into any human cell type, including development from embryo into fetus.

1.2.2.4.1.2: Pluripotential stem cells: These cells are present several days after fertilization. Pluripotent stem cells can develop into any cell type, except they cannot develop into a fetus.

1.2.2.4.1.3: Multipotential stem cell: These cells are derived from pluripotent stem cells. They can be found in adults, but they are limited to specific types of cells to form tissues. For example, bone marrow stem cells can produce all types of blood cells, bone cartilage, and adipose (fat) cells. **(2)**

1.2.2.4.2: Cellular elements of bone marrow:

1.2.2.4.2.1: Progenitor Blood Cells:

The pluripotent stem cell is the first in a sequence of steps of hematopoietic cell generation and maturation. The progenitor of all blood cells is called the multipotential hematopoietic stem cell. Stem cells carry out the ultimate burden of generating multilineage mature blood cells over the lifetime of the organism. During this span of time, the stem cell population may undergo quantitative and qualitative changes .Stem cells have the capacity for self-renewal as well as proliferation and differentiation into progenitor cells. Recent research has demonstrated that blood, brain, and many other regions of the body have their own specialized stem cells that are capable of making replacement cells. Some

of these stem cells are amazingly adaptable, a concept referred to as "stem cell plasticity," and are able to generate an assortment of seemingly unrelated types of cells. This research suggests that adults carry a reservoir of "master cells" inside their bone marrow that are capable of rebuilding almost any damaged tissue. These "master cells" are being called multipotent adult progenitor cells (MAPCs). MAPCs express an enzyme called telomerase that keeps cells from aging. In vitro, MAPCs can be coaxed into becoming muscle, cartilage, bone, liver, or different types of neurons and brain cells Hematopoietic cells can be divided into three phases according to cell maturity:

- Primitive, multipotential cells: The most immature group capable of self-renewal and differentiation into all blood cell lines.

- Intermediate cells: This group consists of committed progenitor cells destined to develop into distinct cell lines.

- Mature cells:The most developed group with specific functions.

The multipotential stem cell is the progenitor of two major ancestral cell lines: lymphocytic and non lymphocytic cells. The lymphoid stem cell is the precursor of either mature Tcells or B cells/plasma cells. The non lymphocytic (myeloid) stem cell progresses to the progenitor colony-forming unit, granulocyte-erythrocyte-monocyte-megakaryocyte (CFUGEMM). The acronym CFU is used as a prefix to record the number of colony-forming units of different progenitor cells that are identified through in vitro clonal assays. The unit colony of CFU-GEMM leads to the development of distinct subsets of committed progenitor cells. The CFU-GEMM can lead to the formation of CFU-granulocyte macrophage/monocyte (CFU-GM), CFU-eosinophil (CFU-Eo), CFU-basophil (CFU-B), and CFU-megakaryocyte (CFU-Meg). In erythropoiesis, the CFU-GEMM differentiates into the burst-forming unit-erythroid(BFU-E). Each of the CFUs in turn can produce a colony of

one hematopoietic lineage under appropriate growth conditions.

The formation and development of mature blood cells from the bone marrow multipotential stem cell is controlled by growth factors and inhibitors as well as the microenvironment. The microenvironment or local influences behavior and controls proliferation of multipotential cells. Bone seems to provide the microenvironment most appropriate for proliferation and maturation of cells. Hematopoietic progenitor cells (HPCs) can be mobilized from the bone marrow to the blood by a wide variety of stimuli, including hematopoietic growth factors and chemokines. Individual hematopoietic cytokines can be lineage specific or can regulate cells in multiple lineages, and for some cell types, e.g. stem cells, the simultaneous action of multiple cytokines is required for proliferative responses. HPCs in the bone marrow exist in a highly organized, three-dimensional microenvironment composed of a diverse population of stromal cells and an extracellular matrix rich in fibronectin , collagens, and various proteoglycans. Hematopoietic progenitor can be found in umbilical cord blood (UCB) as well. UCB hematopoietic cells have been employed successfully as a therapeutic source of autologous and allogeneic transplants for more than 20 years. Cryopreservation prolongs the storage time of UCB. **(2)**

1.2.2.5: Hematopoiesis categories according to cells:

1.2.2.5.1: Leukopoiesis:

Leukopoiesis is a form of hematopoiesis in which white blood cells (WBC or leukocytes) are formed in bone marrow located in bones in adults and hematopoietic organs in the fetus. White blood cells, indeed all blood cells, are formed from the differentiation of pluripotent hematopoietic stem cells which give rise to several cell lines with more limited differentiation potential. These immediate cell lines, or colonies, are progenitors of red blood cells (erythrocytes), platelets

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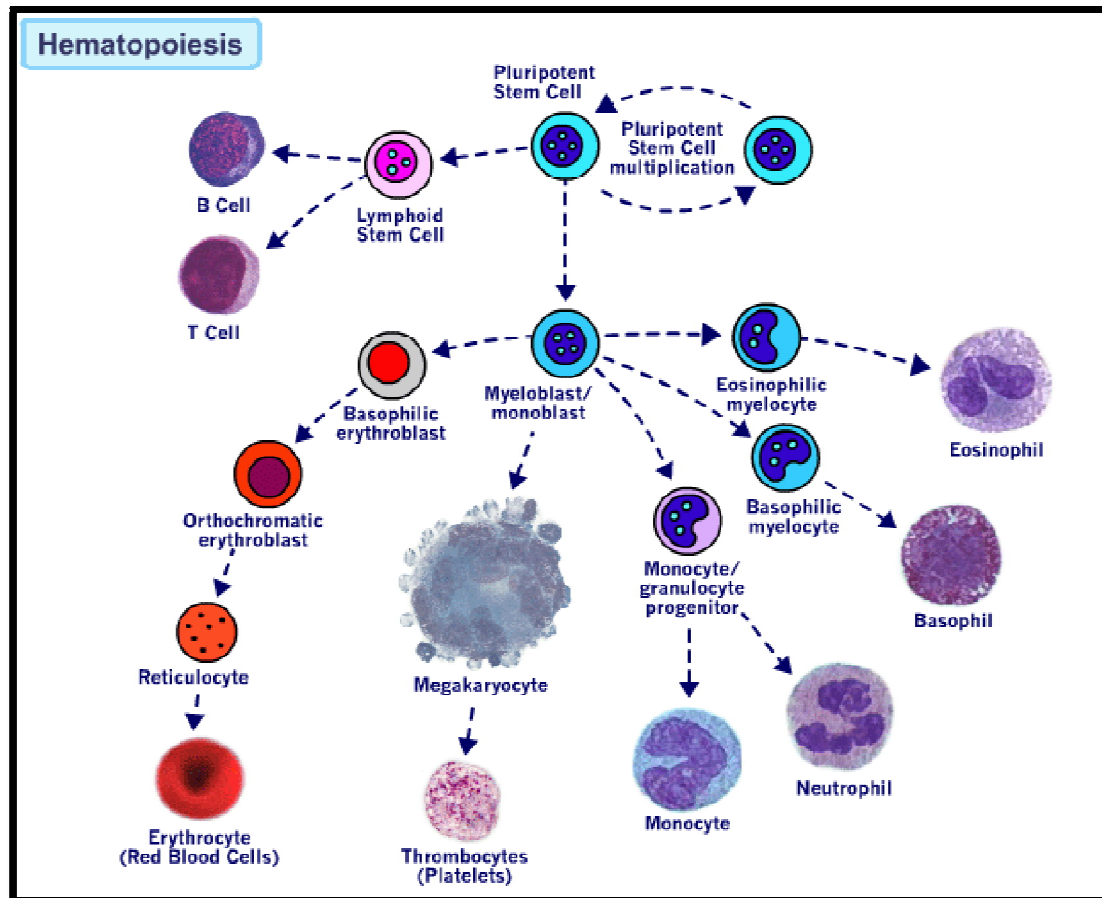(megakaryocytes), and the two main groups of WBCs, myelocytes and lymphocytes.

Figure (1.1): Differentiation of blood cells from stem cell.

1.2.2.5.2: Erythropoiesis:

It occurs in distinct anatomical sites called erythropoietic islands, specialized niches in which erythroid precursors proliferate, differentiate, and enucleate. Each island consists of a macrophage surrounded by a cluster of erythroblasts. Within erythroid niches, cell-cell and cell–extracellular matrix adhesion, positive and negative regulatory feedback, and central macrophage function occur. Erythroid cells account for 5% to 38% of nucleated cells in normal bone. **(2)**

1.2.2.5.3: Granulopoiesis:

Myeloid cells account for 23% to 85% of the nucleated cells in normal bone marrow. Granulopoiesis can be recognized as a maturational unit.

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Early cells are located in the cords and around the bone trabeculae. Neutrophils in the bone marrow reside in the proliferating pool and the maturation storage. Maturing cells spend an average of 3 to 6 days in the proliferating pool. If needed, cells from the storage pool can exit into the circulation rapidly and will have an average life span of 6 to 10 hours. (2)

1.2.2.5.4: Lymphopoiesis:

Unlike other cell lines, lymphocytes and plasma cells are produced in lymphoid follicles. Lymphocytes are randomly dispersed throughout the cords. Lymphoid follicles may also be observed, especially after the age of 50. Plasma cells are located along the vascular wall. Lymphoid cells typically account for 1% to 5% of the nucleated cells in the normal bone marrow. (2)

1.2.2.5.5: Megakaryopoiesis:

is haematopoiesis of megakaryocytes. It takes place adjacent to the sinus endothelium. Megakaryocytes produced through the vascular wall as small cytoplasmic processes to deliver platelets into the sinusoidal blood. Megakaryocytes develop into platelets in approximately 5 days. (2)
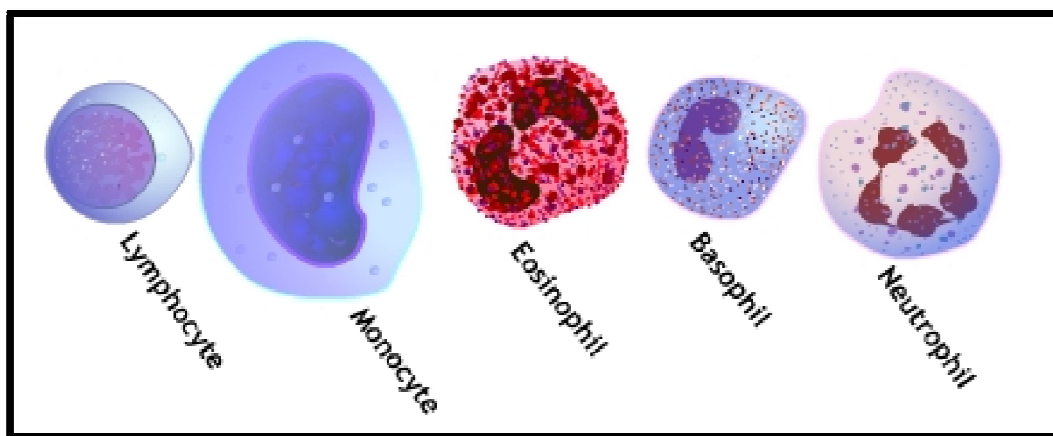

Figure (1.2): Blood cells types.

1.2.3: Normal WBCs:

1.2.3.1: Neurophils:

1.2.3.1.1: Development and functions:

A neutrophil is a granulocyte (a type of white blood cell) that is designed to fight off infections and diseases that enter the body. Neutrophils, along with eosinophils and basophils, are members of the polymorphonuclear cells (PMNs). These cells are filled with neutrally-staining granules, which are small pouches of enzymes that allow the cell to destroy an invading microorganism it has engulfed during phagocytosis. Neutrophils are the most abundant type of white blood cells in the body, making up 70% of all leukocytes (white blood cells). These cells play an important role in the immune system. When a pathogen (disease-causing microorganism) enters the body, neutrophils are the first phagocytes to attack the invader. These cells are the main component of pus, and are responsible for its yellow/white appearance. **(10)**

Neutrophils are produced in the bone marrow. Mature neutrophils are normally found in the bloodstream. However, during inflammation, neutrophils move toward the affected area within an hour by a process known as chemotaxis. Neutrophils are continually produced in the bone marrow. A mature neutrophil has a segmented nucleus, while an immature neutrophil has a band-shaped nucleus. On average, neutrophils typically live about three days. The neutrophil plasma membrane contains several membrane channels, adhesive proteins, receptors for various ligands (molecules that bind to specific proteins), ion pumps and ectoenzymes (enzymes located on the outer surface of the cell). **(10)**

Neutrophils have a complex cytoskeleton, which is responsible for chemotaxis (movement), phagocytosis (engulfing organisms) and exocytosis (secretions released outside of the cell). Proteins that make up the cytoskeleton include actin, actin-binding protein, alpha-actinin,

myosin, tubulin gelsolin, profilin and tropomyosin. About 45% of the neutrophil cytosolic protein is made of migration inhibitory factor-related proteins (MRPs), MRP-8 and MRP-14. Neutrophils contain a large amount of glycogen (a stored form of glucose) in the cytoplasm. The glycogen provides neutrophils with energy. Once fully developed, neutrophils are no longer able to grow or divide. Mature neutrophils contain at least four types of granules, which are specialized lysosomes (particles that contain enzymes necessary for digestion). Granules are classified as, primary or azurophil granules, secondary or specific granules, tertiary or gelatinase granules and secretory vesicles. **(10)**

1.2.3.1.2: Types of granules:

1.2.3.1.2.1: Primary or azurophil granules: Azurophil granules are released into the phagocytic vesicles. They contain an enzyme called myeloperoxidase, as well as several other proteins and enzymes. Myeloperoxidase (MPO) is a catalyst in the conversion of hydrogen peroxide to hypochlorous acid. MPO is responsible for the green color of pus. Other components of azurophilic granules include defensins, lysozyme, azurocidin, bacterial permeability-increasing protein (BPI), elastase, cathepsin G, proteinase and esterase N. Defensins are proteins that fight against bacteria, fungi and viruses. Lysozyme is an enzyme that degrades bacterial peptidoglycans, which are found in the cellular membranes of bacteria. Azurocidin demonstrates antibacterial activity and antifungal activity against Candida albicans. BPI has antibacterial activity against some gram-negative bacteria. **(10)**

1.2.3.1.2.2: Secondary or specific granules: Secondary, or specific granules, are secreted outside of the neutrophil (exocytosis) Secondary granules contain apolactoferrin, vitamin B-12-binding protein, plasminogen activator, lysozyme and collagenase. Apolactoferrin binds to the iron, which deprives bacteria of iron that is needed for cell growth.

The collagenase enzyme breaks down collagen, which allows neutrophils to move freely through collagen.

1.2.3.1.2.3: Tertiary or gelatinase granules: Tertiary, or gelatinase granules, contains gelatinase, acetyltransferase and lysozyme. Tertiary granules, along with specific granules, are up-regulated to the cell surface when they are stimulated. Gelatinase is used to facilitate neutrophil movement through the tissues.

1.2.3.1.2.4: Secretory vesicles: Secretory vesicles contain alkaline phosphatase, cytochrome b558 and N-formyl-1-methionyl-1-leucyl-1-phenylalanine (FMLP) receptors. Secretory vesicles can be up-regulated to the surface, even if extracellular calcium is absent. The FMLP activates neutrophils to engulf foreign invaders when it comes into contact with N-formylated peptides. **(10)**

1.2.3.1.3: Neutrophil maturation stages:

The neutrophils replicate during the first three stages of development, and then undergo cell differentiation during the later stages.

Myeloblast stage: The first stage of neutrophil development, known as the myeloblast stage, occurs in the bone marrow. The myeloblast cell is round, and it has a large nucleus, containing 2-5 nucleoli. There is a small amount of cytoplasm, which does not contain granules. The chromatin in the nucleus is not condensed. **(10)**

1.2.3.1.3.1: Promyelocyte stage: The promyelocyte cell, which is still in the bone marrow, is larger than the myeloblast. The nucleus is round or oval, and the nuclear chromatin is not condensed. As the cell develops, the nucleoli are less prominent. The azurophilic (primary granules) are present in the cytoplast. The primary granules bud off the concave surface of the Golgi complex, an organelle that modifies and creates proteins and other chemicals for use outside the cell. However, the secondary granules have not developed yet. **(10)**

1.2.3.1.3.2: Myelocyte stage: In the myelocyte stage, which takes place in the bone marrow, the secondary granules develop. These granules are smaller than the primary granules, and the glycoprotein is easily seen when the cell is stained. Secondary granules arise from the convex surface of the Golgi complex, an organelle that modifies and creates proteins and other chemicals for use outside the cell. The nucleus at this stage is round or oval, and the chromatin is coarse. The nucleoli are smaller and less prominent than they are in the promyelocyte stage. **(10)** Primary granules only form during the promyelocyte stage. With each cell division, the number of primary granules decreases. In mature neutrophils, the ratio of secondary granules to primary granules in humans is about 2-3:1.

1.2.3.1.3.3: Metamyelocyte stage: The metamyelocytes are present in the blood. The metamyelocyte stage is characterized by an indented nucleus that does not contain nucleoli. The dense chromatin clumps together along the nuclear membrane. The cytoplasm is filled with primary, secondary and tertiary granules. The metamyelocyte stage is not capable of cell division. Instead, cell differentiation takes place. **(10)**

1.2.3.1.3.4: Polymorphonuclear stage: In the last stage, band neutrophils undergo further condensation of the nuclear chromatin. The nucleus is sausage-shaped. The nucleus begins to develop one or more constrictions, and, as the cell develops into the polymorphonuclear stage, the nucleus has two or more lobes connected by filamentous strands. In the polymorphonuclear stage, the cytoplasm appears faintly pink due to an abundance of granules. The fully mature neutrophils are present in the blood and tissues. **(10)**
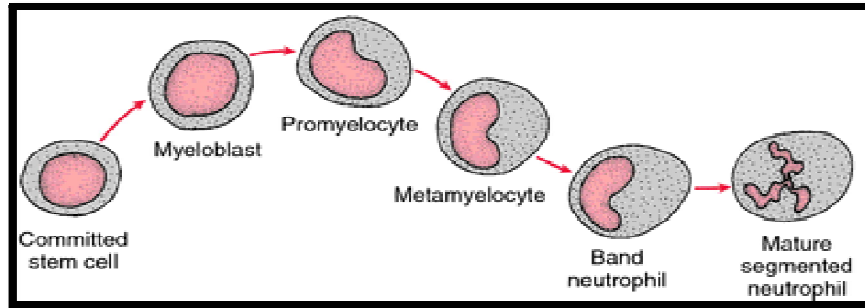
Figure (1.3): Neutrophil maturation stages.

1.2.3.1.4: Neutrophil Functions:

1.2.3.1.4.1: Chemotaxis:

Neutrophils undergo a process called chemotaxis, which allows them to migrate toward sites of infection or inflammation. Cell surface receptors allow neutrophils to detect chemical gradients of molecules such as interleukin-8 (IL-8), interferon gamma (IFN-gamma), C5a, and Leukotriene B4, which these cells use to direct the path of their migration. Neutrophils have a variety of specific receptors, including complement receptors, cytokine receptors for interleukins and interferon gamma (IFN-gamma), receptors for chemokines, receptors to detect and adhere to endothelium, receptors for leptins and proteins, and Fc receptors for opsonin.**[8]**

1.2.3.1.4.2: Anti-microbial function:

Being highly motile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells, and macrophages. Neutrophils express and release cytokines, which in turn amplify inflammatory reactions by several other cell types.

In addition to recruiting and activating other cells of the immune system, neutrophils play a key role in the front-line defence against invading pathogens. Neutrophils have three methods for directly attacking micro-organisms: phagocytosis (ingestion), release of soluble anti-microbials

(including granule proteins), and generation of neutrophil extracellular traps (NETs). **(8)**

1.2.3.1.4.3: Phagocytosis

Neutrophils are phagocytes, capable of ingesting microorganisms or particles. For targets to be recognised, they must be coated in opsonins—a process known as antibody opsonization. They can internalize and kill many microbes, each phagocytic event resulting in the formation of a phagosome into which reactive oxygen species and hydrolytic enzymes are secreted. The consumption of oxygen during the generation of reactive oxygen species has been termed the "respiratory burst", although unrelated to respiration or energy production. The respiratory burst involves the activation of the enzyme NADPH oxidase, which produces large quantities of superoxide, a reactive oxygen species. Superoxide decays spontaneously or is broken down via enzymes known as superoxide dismutases (Cu/ZnSOD and MnSOD), to hydrogen peroxide, which is then converted to hypochlorous acid HClO, by the green heme enzyme myeloperoxidase. It is thought that the bactericidal properties of HClO are enough to kill bacteria phagocytosed by the neutrophil, but this may instead be a step necessary for the activation of proteases. **(8)**

1.2.3.1.4.4: Degranulation:

Neutrophils also release an assortment of proteins in three types of granules by a process called degranulation. The contents of these granules have antimicrobial properties, and help combat infection. **(8)**

1.2.3.1.5: Neutrophil extracellular traps (NETs):

In 2004, Brinkmann and colleagues described a striking observation that activation of neutrophils causes the release of web-like structures of DNA; this represents a third mechanism for killing bacteria. These neutrophil extracellular traps (NETs) comprise a web of fibers composed of chromatin and serine proteases that trap and kill microbes

extracellularly. It is suggested that NETs provide a high local concentration of antimicrobial components and bind, disarm, and kill microbes independent of phagocytic uptake. In addition to their possible antimicrobial properties, NETs may serve as a physical barrier that prevents further spread of pathogens. Trapping of bacteria may be a particularly important role for NETs in sepsis, where NET are formed within blood vessels.Recently, NETs have been shown to play a role in inflammatory diseases, as NETs could be detected in preeclampsia, a pregnancy-related inflammatory disorder in which neutrophils are known to be activated.In addition, NETs are known to exhibit pro-thrombotic effects both in vitro and in vivo. **(8)**

1.2.3.1.6: Role in disease:

Low neutrophil counts are termed neutropenia. This can be congenital (genetic disorder) or it can develop later, as in the case of aplastic anemia or some kinds of leukemia. It can also be a side-effect of medication, most prominently chemotherapy. Neutropenia makes an individual highly susceptible to infections. Neutropenia can be the result of colonization by intracellular neutrophilic parasites. In alpha 1-antitrypsin deficiency, the important neutrophil enzyme elastase is not adequately inhibited by alpha 1-antitrypsin, leading to excessive tissue damage in the presence of inflammation – the most prominent one being pulmonary emphysema. In Familial Mediterranean fever (FMF), a mutation in the pyrin (or marenostrin) gene, which is expressed mainly in neutrophil granulocytes, leads to a constitutively active acute-phase response and causes attacks of fever, arthralgia, peritonitis, and – eventually – amyloidosis. **(10)**

1.2.3.1.7: Neutrophil antigens:

There are five (HNA 1-5) sets of neutrophil antigen recognised.The three HNA-1 antigens (a-c) are located on the low affinity Fc-γ receptor IIIb (FCGR3B:CD16b). The single known HNA-2a antigen is located on

CD177. The HNA-3 antigen system has two antigens (3a and 3b) which are located on the seventh exon of the CLT2 gene (SLC44A2). The HNA-4 and HNA-5 antigen systems each have two known antigens (a) and (b) and are located in the β2 integrin. HNA-4 is located on the αM chain (CD11b) and HNA-5 is located on the αL integrin unit (CD11a). **(10)**

1.2.3.2: Lymphocytes:

1.2.3.2.1: Development and Function:

A lymphocyte is any of 3 types of white blood cell in a vertebrate's immune system. All 3 are agranulocytes. They include natural killer cells (NK cells) (which function in cell-mediated, cytotoxic innate immunity), T cells (for cell-mediated, cytotoxic adaptive immunity), and B cells (for humoral, antibody-driven adaptive immunity). They are the main type of cell found in lymph, which prompted the name lymphocyte. The normal range for lymphocytes in an adult is 22% to 40%, with absolute values of 1.2 to 3.4×10^9/L. The following is a sample calculation of the absolute lymphocyte count:

Absolute number = total leukocyte count $\times$ relative % of lymphocytes

Total leukocyte count = 25.0×10^9/L

Relative number of lymphocytes = 76%

Absolute number = 19.0×10^9/L

Microscopically, in a Wright's stained peripheral blood smear, a normal lymphocyte has a large, dark-staining nucleus with little to no eosinophilic cytoplasm. In normal situations, the coarse, dense nucleus of a lymphocyte is approximately the size of a red blood cell (about 7 micrometres in diameter). Some lymphocytes show a clear perinuclear zone (or halo) around the nucleus or could exhibit a small clear zone to one side of the nucleus. Polyribosomes are a prominent feature in the lymphocytes and can be viewed with an electron microscope. The

ribosomes are involved in protein synthesis, allowing the generation of large quantities of cytokines and immunoglobulins by these cells. (8)

It is impossible to distinguish between T cells and B cells in a peripheral blood smear. Normally, flow cytometry testing is used for specific lymphocyte population counts. This can be used to specifically determine the percentage of lymphocytes that contain a particular combination of specific cell surface proteins, such as immunoglobulins or cluster of differentiation (CD) markers or that produce particular proteins (for example, cytokines using intracellular cytokine staining (ICCS)). In order to study the function of a lymphocyte by virtue of the proteins it generates, other scientific techniques like the ELISPOT or secretion assay techniques can be used. (8)

1.2.3.2.2: Sites of Lymphocytic Development:

During embryonic development, lymphocytes arise from the pluripotent, precursor cells of the yolk sac and liver. Later in fetal development and throughout the life cycle, the bone marrow becomes the sole provider of hematopoietic stem cells. Cells under the influence of hematopoietic growth factors interleukin-1 (IL-1) and IL-6 differentiate into the lymphoid stem cell. Continued cellular development of the lymphoid precursors and proliferation occur as the cells travel to specific microenvironments. Hematopoietic growth factors play an important role in differentiation into the pathway of the pre-B cell or prothymocyte. The majority of cells differentiate into either T lymphocytes or B lymphocytes. The plasma cell is the fully differentiated B cell. (2)

1.2.3.2.2.1: Primary Lymphoid Tissue:

In humans, both the bone marrow and the thymus are classified as primary or central lymphoid tissues and are active in lymphopoiesis. Stem cells that migrate to the thymus proliferate and differentiate under the influence of specific cytokines. These cells acquire thymus-dependent

characteristics to become immunocompetent (able to function in the immune response) T lymphocytes. It is believed that the bone marrow functions as the bursal equivalent in humans. It is from the term bursa that the B lymphocytes derive their name. Most of the cells produced in the primary sites die before leaving; only a small percentage migrate to the secondary tissues. **(2)**

1.2.3.2.2.2: Secondary Lymphoid Tissue:

The secondary lymphoid tissues include the lymph nodes, spleen, and Peyer patches in the intestine .Proliferation of the T and B lymphocytes in the secondary or peripheral lymphoid tissues is primarily dependent on antigenic stimulation. The T lymphocytes or T cells are located in:

- Perifollicular and paracortical regions of the lymph node
- Medullary cords of the lymph nodes
- Periarteriolar regions of the spleen
- Thoracic duct of the circulatory system
- The B lymphocytes or B cells multiply and populate these sites:
- Follicular and medullary areas (germinal centers) of the lymph nodes.
- Primary follicles and red pulp of the spleen.
- Follicular regions of gut-associated lymphoid tissue (GALT).
- Medullary cords of the lymph nodes. **(2)**

1.2.3.2.3: Lymphocyte Maturational Stages:

The stages of lymphocyte development are the lymphoblast, prolymphocyte, and mature lymphocyte. The morphological characteristics of these cells on a peripheral blood smear when stained with Wright stain are under normal conditions, only mature lymphocytes are found in the peripheral blood. Mature cells can be classified as either large or small. Although T and B cells cannot be distinguished by routine Romanowsky-type staining of blood smears, most small lymphocytes are

T cells and most large are B cells.**(4)**

1.2.3.2.3.1: Lymphoblast:

The lymphoblast is the first morphologically identifi able cell of the lymphocytic maturational series in the bone marrow. The overall size ranges from 15 to 20 mm, with a nuclear-cytoplasmic (N: C) ratio of 4:1. The nuclear shape is either round or oval. One or two nucleoli may be present. The chromatin pattern is delicate looking. The small amount of cytoplasm is medium blue and may have a darker-blue border. No granules are present. **(4)**

1.2.3.2.3.2: Prolymphocyte:

The second stage in the maturational development of the lymphocyte is the prolymphocyte. This cell may be seen in the bone marrow, thymus, and secondary lymphoid tissues. The overall size is usually about the same (15 to 18 mm) as the lymphoblast. The N:C ratio ranges from 4:1 to 3:1. The nuclear shape is usually oval or slightly indented. The number of nucleoli varies from none to one. The chromatin pattern is slightly condensed. The small amount of cytoplasm is medium blue with a thin, darker blue rim. A few azurophilic granules may be present. **(4)**

1.2.3.2.3.3: Mature Lymphocyte:

Mature lymphocytes range in size from large (17 to 20 mm) in younger cells to small (6 to 9 mm) in older cells. The N: C ratio ranges from 2:1 in younger cells to 4:1 to 3:1 in older cells. The nucleus is round or oval and may have an indentation (cleft). Nucleoli are not visible. The chromatin pattern is dense and appears clumped. The cytoplasm is light sky blue and very scanty. A few azurophilic granules may be present. Erythrocytes in late stages of nucleated development should not be confused with mature lymphocytes. **(4)**

1.2.3.2.4: General Variations in Lymphocyte Morphology:

Variant lymphocytes may be referred to by several names, including

atypical lymphocytes, Downey cells, reactive or transformed lymphocytes, lymphocytoid or plasmacytoid lymphocytes, and virocytes. The term variant denotes that a lymphocyte is not normal but does not further classify a lymphocyte. Healthy persons may have up to 5% or 6% of variant lymphocytes. These represent morphological evidence of a normal immune mechanism. Variant lymphocytes can be found in increased numbers in disorders such as infectious mononucleosis, viral pneumonia, and viral hepatitis. The morphology of variant lymphocytes differs, and several distinct types have been described, including the classic but obsolete grouping of lymphocytes seen in infectious mononucleosis, the Downey classification. Variant lymphocytes can embrace all transitional changes from mature unstimulated lymphocytes to immunoblasts toplasma cells. These lymphocytes represent stimulated lymphocytes that have increased DNA and RNA activity. Similarities between some variant lymphocytes and lymphoblasts can lead to difficulties in identification. The formation of distinctive nucleoli characterizes the immunoblast, the further transformation of which produces plasma cells or small sensitized committed lymphocytes called memory cells.[2]

The general characteristics of variant lymphocytes include the following:

- Usually the overall size is increased (16 to 30 mm).

- The nucleus may be enlarged.

- The nuclear shape may be lobulated or resemble the nucleus of a monocyte (monocytoid) with clefts or notching and may be folded.

- Chromatin patterns vary from fine patterns to a coarsely granular appearance.

- One to three nucleoli may be present.

- The cytoplasm is frequently abundant and often foamy or vacuolated

- Cytoplasmic color may range from gray to light blue or intensely blue.
- Granules may be present. **(2)**

1.2.3.2.5: Types of Lymphocytes:

The three major types of lymphocyte are T cells, B cells and natural killer (NK) cells. Lymphocytes can be identified by their large nucleus.

1.2.3.2.5.1: T cells and B cells:

T cells (thymus cells) and B cells (bursa-derived cell) are the major cellular components of the adaptive immune response. T cells are involved in cell-mediated immunity, whereas B cells are primarily responsible for humoral immunity (relating to antibodies). The function of T cells and B cells is to recognize specific "non-self" antigens, during a process known as antigen presentation. Once they have identified an invader, the cells generate specific responses that are tailored to maximally eliminate specific pathogens or pathogen-infected cells. B cells respond to pathogens by producing large quantities of antibodies which then neutralize foreign objects like bacteria and viruses. In response to pathogens some T cells, called *T helper cells*, produce cytokines that direct the immune response, while other T cells, called cytotoxic T cells, produce toxic granules that contain powerful enzymes which induce the death of pathogen-infected cells. Following activation, B cells and T cells leave a lasting legacy of the antigens they have encountered, in the form of memory cells. Throughout the lifetime of an animal these memory cells will "remember" each specific pathogen encountered, and are able to mount a strong and rapid response if the pathogen is detected again. The T Helper Cell is a sub-type of the T cell that is able to activate all these lymphocytes. **(8)**

1.2.3.2.5.2: Natural killer cells:

NK cells are a part of the innate immune system and play a major role in defending the host from both tumors and virally infected cells. NK cells

distinguish infected cells and tumors from normal and uninfected cells by recognizing changes of a surface molecule called MHC (histocompatibility complex) class I. NK cells are activated in response to a family of cytokines called interferons. Activated NK cells release cytotoxic (cell-killing) granules which then destroy the altered cells. They were named "natural killer cells" because of the initial notion that they do not require prior activation in order to kill cells which are missing MHC class I. (8)

1.2.3.3: Monocytes:

1.2.3.3.1: Development and Function:

Monocytes are a type of white blood cells (leukocytes). They are the largest of all leukocytes. They are part of the innate immune system of vertebrates including all mammals (humans included), birds, reptiles, and fish. They are amoeboid in shape, having clear cytoplasm. Monocytes have bean-shaped nuclei that are unilobar, which makes them one of the types of mononuclear leukocytes (agranulocytes). Monocytes constitute 2% to 10% of all leukocytes in the human body. They play multiple roles in immune function. Such roles include: (1) replenishing resident macrophages under normal states, and (2) in response to inflammation signals, monocytes can move quickly (approx. 8–12 hours) to sites of infection in the tissues and divide/differentiate into macrophages and dendritic cells to elicit an immune response. Half of them are stored in the spleen (except in people who have undergone splenectomy). Monocytes are usually identified in stained smears by their large kidney shaped or notched nucleus. These change into macrophages after entering into the tissue spaces. (8)

1.2.3.3.2: Physiology:

Monocytes are produced by the bone marrow from precursors called monoblasts, bipotent cells that differentiated from hematopoietic stem cells. Monocytes circulate in the bloodstream for about one to three days and then typically move into tissues throughout the body. They constitute between three to eight percent of the leukocytes in the blood. Half of them are stored as a reserve in the spleen in clusters in the red pulp's Cords of Billroth. In the tissues, monocytes mature into different types of macrophages at different anatomical locations. Monocytes are the largest corpuscles in the blood. Monocytes which migrate from the bloodstream to other tissues will then differentiate into tissue resident macrophages or dendritic cells. Macrophages are responsible for protecting tissues from foreign substances, but are also suspected to be important in the formation of important organs like the heart and brain. They are cells that possess a large smooth nucleus, a large area of cytoplasm, and many internal vesicles for processing foreign material. **(8)**

Monocytes and their macrophage and dendritic-cell progeny serve three main functions in the immune system. These are phagocytosis, antigen presentation, and cytokine production. Phagocytosis is the process of uptake of microbes and particles followed by digestion and destruction of this material. Monocytes can perform phagocytosis using intermediary (opsonising) proteins such as antibodies or complement that coat the pathogen, as well as by binding to the microbe directly via pattern-recognition receptors that recognize pathogens. Monocytes are also capable of killing infected host cells via antibody, termed antibody-mediated cellular cytotoxicity. Vacuolization may be present in a cell that has recently phagocytized foreign matter. Microbial fragments that remain after such digestion can serve as antigens. The fragments can be incorporated into MHC molecules and then trafficked to the cell surface

of monocytes (and macrophages and dendritic cells). This process is called antigen presentation and it leads to activation of T lymphocytes, which then mount a specific immune response against the antigen.(8)

1.2.3.3.3: Monocyte subpopulations:

There are at least three types of monocytes in human blood:

a) The classical monocyte is characterized by high level expression of the CD14 cell surface receptor (CD14++ CD16- monocyte)

b) The non-classical monocyte shows low level expression of CD14 and additional co-expression of the CD16 receptor (CD14+CD16++ monocyte).

c) The intermediate monocyte with high level expression of CD14 and low level expression of CD16 (CD14++CD16+ monocytes).

There appears to be a developmental relationship in that the classical monocytes develop into the intermediate monocytes to then become the non-classical CD14+CD16++ monocytes. Hence the non-classical monocytes may represent a more mature version. After stimulation with microbial products the CD14+CD16++ monocytes produce high amounts of pro-inflammatory cytokines like tumor necrosis factor and interleukin-12. Said et al. showed that activated monocytes express high levels of PD-1 which might explain the higher expression of PD-1 in CD14+CD16++ monocytes as compared to CD14++CD16- monocytes. Triggering monocytes-expressed PD-1 by its ligand PD-L1 induces IL-10 production which activates CD4 Th2 cells and inhibits CD4 Th1 cell function. (8)

1.2.3.4: Eosinophils:

1.2.3.4.1: Development and Function:

Eosinophil granulocytes, usually called **eosinophils** or **eosinophiles** (or, less commonly, acidophils), are white blood cells and one of the immune system components responsible for combating multicellular parasites and

certain infections in vertebrates. Along with mast cells, they also control mechanisms associated with allergy and asthma. They are granulocytes that develop during hematopoiesis in the bone marrow before migrating into blood. These cells are eosinophilic or 'acid-loving' as shown by their affinity to coal tar dyes: Normally transparent, it is this affinity that causes them to appear brick-red after staining with eosin, a red dye, using the Romanowsky method. The staining is concentrated in small granules within the cellular cytoplasm, which contain many chemical mediators, such as histamines and proteins such as eosinophil peroxidase, ribonuclease (RNase), deoxyribonucleases, lipase, plasminogen, and major basic protein. These mediators are released by a process called degranulation following activation of the eosinophil, and are toxic to both parasite and host tissues. In normal individuals, eosinophils make up about 1-6% of white blood cells, and are about 12-17 micrometers in size. They are found in the medulla and the junction between the cortex and medulla of the thymus, and, in the lower gastrointestinal tract, ovary, uterus, spleen, and lymph nodes, but not in the lung, skin, esophagus, or some other internal organs under normal conditions. The presence of eosinophils in these latter organs is associated with disease. Eosinophils persist in the circulation for 8–12 hours, and can survive in tissue for an additional 8–12 days in the absence of stimulation. Pioneering work in the 1980s elucidated that eosinophils were unique granulocytes, having the capacity to survive for extended periods of time after their maturation as demonstrated by ex-vivo culture experiments. **(8)**

1.2.3.4.2: Eosinophil migration and activation:

Eosinophils develop and mature in the bone marrow. They differentiate from myeloid precursor cells in response to the cytokines interleukin 3 (IL-3),interleukin5(IL-5),and granulocyte macrophage-colony stimulating factor (GM-CSF). Eosinophils produce and store many secondary granule

proteins prior to their exit from the bone marrow. After maturation, eosinophils circulate in blood and migrate to inflammatory sites in tissues, or to sites of helminth infection in response to chemokines like CCL11 (eotaxin-1), CCL24 (eotaxin-2), CCL5 (RANTES), and certain leukotrienes like leukotriene B4 (LTB4) and MCP1/4. At these infectious sites, eosinophils are activated by Type 2 cytokines released from a specific subset of helper T cells (T_h2); IL-5, GM-CSF, and IL-3 are important for eosinophil activation as well as maturation. There is evidence to suggest that eosinophil granule protein expression is regulated by the non-coding RNA EGOT (gene). **(8)**

1.2.3.4.3: Functions of eosinophils:

Following activation, eosinophils effector functions include production of:

* Cationic granule proteins and their release by degranulation.
* Reactive oxygen species such as hypobromite, superoxide, and peroxide (hypobromous acid, which is preferentially produced by eosinophil peroxidase).
* lipid mediators like the eicosanoids from the leukotriene (e.g., LTC_4, LTD_4, LTE_4) and prostaglandin (e.g., PGE_2) families.
* Enzymes, such as elastase.
* cytokines such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, and TNF alpha. **(8)**

  In addition, there are also eosinophils that play a role in fighting viral infections, which is evident from the abundance of RNases they contain within their granules, and in fibrin removal during inflammation. Eosinophils along with basophils and mast cells, are important mediators of allergic responses and asthma pathogenesis and are associated with disease severity. They also fight helminth

(worm) colonization and may be slightly elevated in the presence of certain parasites. Eosinophils are also involved in many other biological processes, including postpubertal mammary gland development, oestrus cycling, allograft rejection and neoplasia. They have also recently been implicated in antigen presentation to T cells. [8]

1.2.3.4.4: Eosinophil granuler proteins:

Following activation by an immune stimulus, eosinophils degranulate to release an array of cytotoxic granule cationic proteins that are capable of inducing tissue damage and dysfunction. These include:

- major basic protein (MBP)
- eosinophil cationic protein (ECP)
- eosinophil peroxidase (EPO)
- eosinophil-derived neurotoxin (EDN)

protein, eosinophil peroxidase, and eosinophil cationic Major basic protein are toxic to many tissues. Eosinophil cationic protein and eosinophil-derived neurotoxin are ribonucleases with antiviral activity. Major basic protein induces mast cell and basophil degranulation, and is implicated in peripheral nerve remodelling. Eosinophil cationic protein creates toxic pores in the membranes of target cells allowing potential entry of other cytotoxic molecules to the cell, can inhibit proliferation of T cells, suppress antibody production by B cells, induce degranulation by mast cells, and stimulate fibroblast cells to secrete mucus and glycosaminoglycan. Eosinophil peroxidase forms reactive oxygen species and reactive nitrogen intermediates that promote oxidative stress in the target, causing cell death by apoptosis and necrosis. [8]

1.2.3.5: Basophils

1.2.3.5.1: Development and Function:

Basophil granulocytes, mostly referred to as basophils, are the least common of the granulocytes, representing about 0.01% to 0.3% of circulating white blood cells. The name comes from the fact that these leukocytes are basophilic, i.e., they are susceptible to staining by basic dyes, as shown in the picture. Basophils contain large cytoplasmic granules which obscure the cell nucleus under the microscope. However, when unstained, the nucleus is visible and it usually has 2 lobes. The mast cell, a cell in tissues, has many similar characteristics. For example, both cell types store histamine, a chemical that is secreted by the cells when stimulated in certain ways (histamine causes some of the symptoms of an allergic reaction). Like all circulating granulocytes, basophils can be recruited out of the blood into a tissue when needed. Basophils appear in many specific kinds of inflammatory reactions, particularly those that cause allergic symptoms. Basophils contain anticoagulant heparin, which prevents blood from clotting too quickly. They also contain the vasodilator histamine, which promotes blood flow to tissues. They can be found in unusually high numbers at sites of ectoparasite infection, e.g., ticks. Like eosinophils, basophils play a role in both parasitic infections and allergies. They are found in tissues where allergic reactions are occurring and probably contribute to the severity of these reactions. Basophils have protein receptors on their cell surface that bind IgE, an immunoglobulin involved in macroparasite defense and allergy. It is the bound IgE antibody that confers a selective response of these cells to environmental substances, for example, pollen proteins or helminth antigens. Recent studies in mice suggest that basophils may also regulate the behavior of T cells and mediate the magnitude of the secondary immune response. **(8)**

1.2.3.5.2: Immunophenotyping of basophils:

Basophils of mouse and human have consistent immunophenotypes as follows: $FcεRI^+$, CD123, $CD49b(DX-5)^+$, $CD69^+$, $Thy-1.2^+$, $2B4^+$, $CD11b^{dull}$, $CD117(c-kit)^-$, $CD24^-$, $CD19^-$, $CD80^-$, $CD14^-$, $CD23^-$, $Ly49c^-$, $CD122^-$, $CD11c^-$, $Gr-1^-$, $NK1.1^-$, $B220^-$, $CD3^-$, $γδTCR^-$, $αβTCR^-$, $α_4$ and $β_4$-integrin negative. Recently, Heneberg proposed that basophils may be defined as the cellular population positive for CD13, CD44, CD54, CD63, CD69, CD107a, CD123, CD164, CD193/ CCR3, CD203c, TLR-4, and FcεRI. When activated, some additional surface markers are known to be upregulated (CD13, CD107a, CD164), or surface-exposed (CD63, and the ectoenzyme CD203c). **(8)**

1.2.3.5.3: Degranulation of basophils:

When activated, basophils degranulate to release histamine, proteoglycans (e.g. heparin and chondroitin), and proteolytic enzymes (e.g. elastase and lysophospholipase). They also secrete lipid mediators like leukotrienes, and several cytokines. Histamine and proteoglycans are pre-stored in the cell's granules while the other secreted substances are newly generated. Each of these substances contributes to inflammation. Recent evidence suggests that basophils are an important source of the cytokine, interleukin-4, perhaps more important than T cells. Interleukin-4 is considered one of the critical cytokines in the development of allergies and the production of IgE antibody by the immune system. There are other substances that can activate basophils to secrete which suggests that these cells have other roles in inflammation.The degranulation of basophils can be investigated in vitro by using flow cytometry and the so-called basophil-activation-test (BAT). Especially, in the diagnosis of allergies including of drug reactions (e.g. induced by contrast medium), the BAT of is great impact. **(8)**

1.2.4: White Blood cells Disorders:

1.2.4.1: Neutrophils Disorders:

1.2.4.1.1: Quantitative disorders:

1.2.4.1.1.1: Neutrophilia:

 Neutrophilia occurs when there are an increased number of neutrophils in the blood. The number of neutrophils may be increased five to six times more than normal. Neutrophilia is common during acute bacterial infections. During early infection, the neutrophil count may actually decrease briefly because early during inflammation, the white blood cells tend to occupy the periphery of the blood vessels. This is followed by a rapid increase of neutrophils from the bone marrow. During the infection, the neutrophil count remains elevated. During the recovery phase, the flow of cells from the marrow decreases, with a resultant decrease in neutrophilia. Other causes of neutrophilia may include rapidly growing, cancerous tumors, acute or chronic administration of corticosteroids and acute hemorrhage (internal bleeding). Neutrophilia has also been associated with Cushing's disease**.**neutrophils can be present in some forms of leukemia and nonmalignant conditions, such as infl ammatory conditions or infection. Neutrophilia can also be caused by physical stimuli such as heat and cold, surgery, burns, stressful activities such as vigorous exercise, nausea, and vomiting. In addition, some drugs and hormones may produce neutrophilia. Some individuals may experience shift neutrophilia, which is usually temporary (lasting about 20-30 minutes). Shift neutrophilia may occur as a result of vigorous exercise or epinephrine injection. It also occurs during seizures and paroxysmal tachycardia. **(11**)

Symptoms: Symptoms vary depending on the underlying cause of neutrophilia. For instance, patients who experience neutrophilia as a result of bacterial infections may have symptoms like inflammation,

enlarged lymph nodes, fever and chills. Patients who experience neutrophilia as a result of cancerous tumors may have symptoms like localized swelling, enlarged lymph nodes or unexplained weight gain or weight loss. Diagnosis: A complete blood count (CBC) test is usually conducted to determine how many and what types of cells are in the blood. The blood test will indicate if there are an increased number of neutrophils in the blood stream. **(11)**

1.2.4.1.1.2: Neutropenia:

It's defined by a low number of neutrophils. In a normal body, neutrophils make up 50-60% of circulating white blood cells, which serve as the body's primary defense mechanism against infection and disease. Neutropenic patients have an absolute neutrophil count (ANC) that is lower than 1,500 cells per micro liter of blood. **(11)**

Therefore, patients with neutropenia are more susceptible to disease and infection. The condition may even become life threatening. Mild neutropenia usually causes no symptoms. Severe neutropenia increases the risk of infection of the lungs, kidneys, blood and skin. Neutropenia can be acute (lasting less than three months) or chronic (lasting longer than three months).Causes of neutropenia include underproduction of cells caused by bone marrow injury or infi ltration of the marrow by malignant cells as well as nutritional deficiencies (such as those caused by starvation or anorexia nervosa). Other causes include cyclic neutropenia, a hereditary disorder; increased destruction or utilization of neutrophils; and entrapment in the spleen.Most transient neutropenias in children are acquired disorders, and viral infections are a common cause. Congenital neutropenia can be caused by a variety of conditions. A rare congenital disorder of young children is congenital agranulocytosis of the Kostmann type. Another uncommon congenital disorder is myelokathexis, the inability to release mature granulocytes into the blood.

Other rare causes of congenital neutropenia include reticular dysgenesis, type IB glycogen storage disease, and transcobalamin-II deficiency. **(11)** Symptoms: Some neutropenic patients, especially those with acute neutropenia, may be asymptomatic or experience mild symptoms. The disease is usually discovered when a patient has developed severe infections or sepsis. Common symptoms of neutropenia include skin rash, mouth ulcers, abscesses, thrush, periodontal disease, lymphadenopathy (enlarged lymph nodes), mucous membrane abnormalities, fever, frequent infections, diarrhea, burning sensation when urinating, unusual redness/pain/swelling around a wound, sore throat, shortness of breath and chills. Diagnosis: Some patients may be asymptomatic or experience mild symptoms. A complete blood count (CBC) test is usually conducted to determine how many and what types of cells are in the blood. Neutropenic patients have an absolute neutrophil count (ANC) that is lower than 1,500 cells per microliter of blood. In serious cases, a bone marrow biopsy may be performed. During the biopsy, the patient is given a local anesthetic, and a sample of bone marrow is removed with a needle. The sample is then analyzed in the laboratory to determine whether or not the bone marrow is producing a sufficient number of neutrophils. Prevention: Neutropenia is a common side effect of chemotherapy in cancer patients. According to the 2006 recommendations from the American Society of Clinical Oncology, blood cell growth factors should be used to prevent febrile neutropenia when the risk of febrile neutropenia is 20% or higher. Recombinant G-CSF (granulocyte-colony stimulating factor) or G-CSF (granulocytes colony-stimulating factor) has been used to stimulate white cell blood production.However, some patients have developed leukemia or myelodysplastic syndrome following treatment with G-CSF. **(11)**

1.2.4.1.2: Qualitative Disorders of Neutrophil:

1.2.4.1.2.1: Defective Locomotion and Chemotaxis:

It represents qualitative defects. Leukocyte mobility may be impaired in diseases such as rheumatoid arthritis, cirrhosis of the liver, and chronic granulomatous disease (CGD). Defective locomotion or leukocyte immobility can be seen in patients receiving corticosteroids and in lazy leukocyte syndrome.A significant defect in the cellular response to chemotaxis is seen in patients who have diabetes mellitus, Chédiak-Higashianomaly, and sepsis as well as in patients with high levels of antibody IgE, such as those with Job syndrome. Abnormalities of mature granulocytes, particularly neutrophils, can be observed in stained smears of peripheral blood. These conditions include the more frequently observed disorders of toxic granulation, Döhle bodies, and hypersegmentation as well as rarely observed disorders such as Pelger-Huët anomaly, May-Hegglin anomaly, Chédiak-Higashi syndrome, and Alder-Reilly inclusions.[2]

1.2.4.1.2.2: Toxic Granulation:

This is a condition in which prominent dark granulation, either fine or heavy, can be observed in band and segmented neutrophils or monocytes .Toxic granules areazurophilic (primary) granules that are peroxidase-positive. The granulation may represent the precipitation of ribosomal protein (RNA) caused by metabolic toxicity within the cells. The extent of toxic granulation is usually graded on a scale of 1+ to 4+, with 4+ being the most severe. Grading of the granulation is dependent on the coarseness and amount of granulation within the cellular cytoplasm. This condition is most frequently associated with infectious states. It may be seen in conditions such as burns and malignant disorders or as the result of drug therapy. [7]

1.2.4.1.2.3: Döhle Bodies:

These inclusion bodies are seen as single or multiple, light blue– staining inclusions on Wright-stained blood smear. They are usually seen near the periphery of the cytoplasm. These inclusions are predominantly seen in neutrophils, although they may be seen in monocytes or lymphocytes. Döhle bodies represent aggregates of rough endoplasmic reticulum (RNA) and may be associated with a variety of conditions such as viral infections, burns, or certain drugs. Döhle body–like inclusions may be seen in May-Hegglin anomaly. **(7)**

1.2.4.1.2.4: Hypersegmentation:

Is most frequently seen in segmented neutrophils with more than five lobes or nuclear segments. This condition is frequently associated with deficiencies of vitamin B12 or folic acid and exists along with abnormal enlarged, oval-shaped erythrocytes. Pseudohypersegmentation may be seen in old segmented neutrophils. **(7)**

1.2.4.1.2.5: Pelger-Huët Anomaly:

This genetically acquired, autosomal dominant disorder produces hyposegmentation of many of the mature neutrophils .The nuclear shape may resemble a dumbbell or a pair of eyeglasses. Although the segments fail to lobulate normally, other characteristics, such as chromatin clumping and cytoplasmic maturation, are normal. Heavy chromatinclumping distinguishes Pelger-Huët anomaly from the left shift of infection. Abnormal nuclear maturation is presumed to be a reflection of abnormal nucleic acid metabolism, although the specific abnormality is unknown. A pseudoanomaly may be drug induced or may occur in a maturational arrest associated with some acute infections. The function of the cell is considered to be normal despite the morphological abnormality. Therefore, it is considered to be a benign anomaly. **(7)**

1.2.4.1.2.6: May-Hegglin Anomaly:

This genetic condition is characterized by the presence of Döhle body–like inclusions in neutrophils, eosinophils, and monocytes .Abnormally large and poorly granulated platelets and thrombocytopenia (a decreased number of platelets) frequently coexist in this condition. Although approximately 50% of patients do not have symptoms; others have manifested abnormal bleeding tendencies. The cause of the hemostatic defect is unclear, but it is proportionate to the degree of thrombocytopenia. (7)

1.2.4.1.2.7: Chédiak-Higashi Syndrome:

This rare disorder is a hereditary disease (autosomal recessive trait). It is primarily seen in children and young adults and is characterized by very large granules .These gigantic, peroxidase-positive deposits represent abnormal lysosomal development in neutrophils and other leukocytes, such as monocytes and lymphocytes. Neutrophils display impaired chemotaxis and delayed killing of ingested bacteria. Patients with this disorder suffer from frequent infections. (7)

1.2.4.1.2.8: Alder-Reilly Inclusions:

These purple-red particles are precipitated mucopolysaccharides

seen primarily in neutrophils, eosinophils, and basophils.Occasionally, they are seen in monocytes and lymphocytes. These inclusions can resemble very coarse toxic granulation. Alder-Reilly granules are most commonly seen in patients with Hurler, Hunter, and Maroteaux-Lamy types of genetic muco-polysaccharidosis. Most of these disorders are transmitted as autosomal recessive genes. (7)

1.2.4.1.2.9: Chronic Granulomatous Disease

CGD is the most serious disorder related to a defect in microbicidal activity. It consists of a group of genetic disorders in which neutrophils and monocytes ingest, but cannot kill, catalase-positive microorganisms

such as Staphylococcus aureus, Gram-positive enteric bacteria, and various fungi, especially Aspergillus. CGD is a rare disorder; the inability to kill microorganisms leads to recurrent life-threatening infections by catalase-positive organisms during the 1st year of life. In CGD, stimulated phagocytes do not generate O2 produce H2O2, or consume O2 at an accelerated rate via the hexose monophosphate (HMP) shunt; the respiratory burst is not activated, and free radical forms of reduced O2 are not produced. In many patients with CGD, the disease is X-linked, but in about one fourth of families, the disease is transmitted by autosomal recessive genes. In most of these cases, both parents have had normal neutrophil functions and their cytochrome *b* concentrations are normal, unlike the X-linked cases. Abnormal oxidase activity is detectable by negative nitroblue tetrazolium (NBT) screening test, an indirect test for respiratory burst power. In addition to the two main categories of CGD, X-linked and the autosomal recessive forms, some cases do not conform to either classification. These cases are believed to be caused by point mutations. Rare causes of CGD include severe deficiency or instability of leukocyte glucose-6-phosphate dehydrogenase (G6PD). (7)

1.2.4.1.2.10: Myeloperoxidase Deficiency:

MPO deficiency (Alius-Grignaschi anomaly) is a benign inherited disorder that is usually transmitted by autosomal recessive genes. This disorder is manifested by the absence of MPO enzyme from neutrophils and monocytes, but not eosinophils. A lack of MPO, which mediates oxidative destruction of microbes by H2O2, creates a microbicidal defect in phagocytes. The functional abnormality is not severe. Infections are not usually serious. A partial deficiency of MPO has been observed in patients with acute and chronic leukemias, myelodysplastic syndromes, Hodgkin disease, and carcinoma. (7)

1.2.4.1.3: Other functional anomalies of neutrophil:

At least 15 hereditary defects and 30 additional disorders of neutrophil function have been described. A functional anomaly of neutrophils includes lactoferrin deficiency. Lactoferrin deficiency is a rare disorder. In this disorder, specific granules are reduced in quantity and almost devoid of the specific granule protein, lactoferrin. This deficiency causes several dysfunctions including unresponsiveness to chemotactic signals and diminished adhesiveness to surfaces of particles. This deficiency leads to pyogenic infections, particularly deep-seated skin abscesses. **(2)**

1.2.4.2: Disorders of lymphocyte:

Disorders of lymphocytes are frequently encountered in the clinical laboratory. Many of these nonmalignant disorders result from viral or bacterial infections. Examples of viral diseases include infectious mononucleosis, cytomegalovirus (CMV) infection, and acquired immunodeficiency syndrome (AIDS). Bacterial diseases associated with lymphocytic disorders can include whooping cough. The parasitic infection toxoplasmosis, although rarer than viral and bacterial causes, can also display lymphocytic involvement. In addition, conditions such as drug-induced (immunological) hypersensitivity reactions elicit lymphocytic proliferative reactions that simulate or even surpass the lymphocytosis observed in infectious mononucleosis. **(2)**

1.2.4.2.1: Lymphocytosis:

Lymphocytosis is natural and normal in infants and children up to approximately 10 years old, with total lymphocyte counts as high as 9 × 109/L. This increase probably results from the limited production of adrenal corticosteroid hormones during this period of the life cycle. This limited production of hormones may underlie the lymphocytosis seen in later childhood in conditions such as malnutrition and scurvy. Lymphocytosis is not a common nonspecific response to inflammation as

is neutrophilia. In adolescence and adulthood, nonmalignant conditions associated with an absolute lymphocytosis include:

- Acute viral infections (e.g., infectious mononucleosis, infectious hepatitis, post transfusion syndrome, CMV infection, and infectious lymphocytosis)
- Some bacterial infections (e.g., Bordetella pertussis infection
- [whooping cough] and brucellosis)
- Parasitic infections (e.g., toxoplasmosis)
- Drug reactions (e.g., p-aminosalicylic acid hypersensitivity and phenytoin hypersensitivity)
- Uncommon causes (e.g., tertiary and congenital syphilis and smallpox)
- Malignant conditions that produce lymphocytosis include:
- Lymphocytic leukemia (acute and chronic forms)
- The leukemic phase of lymphomas
- Waldenström macroglobulinemia. (2)

1.2.4.2.1.1: Disorders associated with lymphocytosis:

1.2.4.2.1.1.1: Infectious Mononucleosis:

IM is a common disease, due to EB virus infection. The hematological features include absolute lymphocytosis with characteristic atypical lymphocytes and positive heterophile antibodies. Associated features can be thrombocytopenia and autoimmune hemolytic anemia. Many other conditions mimic IM in many respects, such as CMV infection, toxoplasmosis, cat-scratch disease; the notable feature is that they are all negative for heterophile antibodies, and the classic atypical lymphocytes are seldom found. **(12)**

1.2.4.2.1.1.2: Cytomegalovirus Infection:

Human CMV is classified as a member of the herpes family of viruses.

There are currently five recognized human herpes viruses: herpes simplex I, herpes simplex II, varicellazoster virus, EBV, and CMV. All the herpesviruses are relatively large, enveloped DNA viruses that undergo a replicative cycle involving DNA expression and nucleocapsid assembly within the nucleus. The viral structure gains an envelope when the virus buds through the nuclear membrane that is altered to contain specific viral proteins. Although the herpes family produces diverse clinical diseases, the viruses share the basic characteristic of being cell associated. The requirements for cell association vary, but all five viruses may spread from cell to cell, presumably via intercellular bridges and in the presence of antibody in the extracellular phase. This common characteristic may play a role in the ability of the virus to produce subclinical infections that can be reactivated under appropriate stimuli. (2)

1.2.4.2.1.1.3: Toxoplasmosis:

The microorganism Toxoplasma gondii causes toxoplasmosis. Toxoplasma was recently recognized as a tissue Coccidia. (2)

1.2.4.2.1.1.4: Infectious Lymphocytosis:

Acute infectious lymphocytosis is a poorly defined benign condition.

Infectious lymphocytosis is caused by a virus, probably a member of the Coxsackie group. Leukocytosis with lymphocytosis characterizes this disease. (2)

1.2.4.2.1.1.5: Bordetella Pertussis (Haemophilus Pertussis) infection:

Whooping cough is caused by B. pertussis, a bacterial organism that produces inflammation of the entire respiratory tract. The total leukocyte count can be increased to as high as 100×109/L.

1.2.4.2.2: Lymphocytopenia:

Lymphocytopenia is generally defined as less than 3.0×109/L lymphocytes in adults or less than 1.5×109/L lymphocytes in children. A decrease in lymphocytes is a common response to stress and to the

administration of corticosteroids, or it may be seen in healthy persons with no apparent cause. Transient relative lymphocytopenia is generally associated with conditions resulting in granulocytosis. Pathological conditions that exhibit absolute lymphocytopenia are related to decreased production, mechanical loss, increased destruction, and various functional abnormalities. These conditions may be caused by immune deficiency disorders, physical agents (e.g., radiation exposure), or cytotoxic drugs.

1.2.4.2.2.1: Immune disorders associated with Lymphocytopenia:

Immune disorders may be caused by defects in the numbers or functional properties of lymphocytes and may be congenital or acquired. These conditions are usually classified as either T-cell or B-cell disorders. Some of the less common disorders involve both T and B cells. **(2)**

1.2.4.2.2.1.1: Di George Syndrome:

A number of T- and B-cell defects involve the alteration of some lymphocyte subpopulations. Patients with Di George syndrome exhibit a decrease in total T lymphocytes coupled with an increased ratio of helper to suppressor cells. In AIDS, a reversed phenotypic helper-to-suppressor ratio due to a decrease in helper cells is observed. A decrease in total T cells and a lack of, or reduced, suppressor cell population are among the immunological changes observed in active SLE. **(2)**

1.2.4.2.2.1.2: Acquired Immunodeficiency Syndrome:

The human immunodeficiency virus (HIV) is the predominant virus responsible for AIDS. Although HIV was recently recognized, it is tentatively concluded that HIV-1 has infected humans for more than 20 but less than 100 years. Laboratory evaluation of HIV-1–infected patients consists of an assessment of cellular and humoral components. Screening of blood donors and patients at risk is usually by serological methods. In patients who have developed the signs and symptoms of AIDS, assessment of the number of lymphocytes and their function becomes

important. Both leukopenia and lymphocytopenia exist in AIDS patients. The number of circulating lymphocytes is severely decreased. **(2)**

1.2.4.2.2.1.3: Systemic Lupus Erythematosus:

SLE is a classic model of autoimmune disease that can affect practically every organ of the body. SLE is a systematic rheumatic disorder, a name commonly used for the disorders of the joints, connective tissues, and collagen-vascular disorders.SLE occurs primarily in adolescent and young adult females and may be present for years before a diagnosis is made. This disorder is eight times more common in female than in male patients. **(2)**

1.2.4.2.2.1.4: Infectious Mononucleosis:

Infectious mononucleosis is usually an acute, benign, and self-limiting lymphoproliferative condition caused by Epstein-Barr virus (EBV). EBV is also the cause of Burkitt lymphoma, a malignant tumor of the lymphoid tissue occurring mainly in African children; nasopharyngeal carcinoma; and neoplasms of the thymus, parotid gland, and supraglottic larynxctious. **(2)**

1.2.4.3: Disorders of Monocytes (monocytosis and monocytopenia):

1.2.4.3.1: Monocytosis:

Monocytosis is the state of excess monocytes in the peripheral blood. It may be indicative of various disease states. Examples of processes that can increase a monocyte count include:

- chronic inflammation
- stress response
- Cushing's syndrome (hyperadrenocorticism)
- immune-mediated disease
- pyogranulomatous disease
- necrosis

- red blood cell regeneration

- Viral Fever

- sarcoidosis

- A high count of CD14+CD16++ monocytes is found in severe infection (sepsis) and a very low count of these cells is found after therapy with immuno-suppressive glucocorticoids.**(8)**

1.2.4.3.2: Monocytopenia:

Monocytopenia is a form of leukopenia associated with a deficiency of monocytes. Can occur in response to the release of toxins into the blood by certain types of bacteria (endotoxemia), as well as in people receiving chemotherapy. **(9)**

1.2.4.4: Disorders of Eosinophils:

1.2.4.4.1: Eosinophilia:

Persistently and significantly increased numbers of eosinophils are most frequently observed in active allergic disorders, such as asthma and hay fever. Other causes of eosinophilia include dermatoses, nonparasitic infections, some forms of leukemia, and parasitic infections. Patients with signifi canteosinophilia usually demonstrate some abnormal morphology. Vacuolization and degranulation can be observed. Charcot-Leyden crystals can be found in the tissues, exudates, sputum, and stool of patients with active eosinophilic inflammation. Eosinophilia is an index of host reaction to parasites and varies considerably from one patient to another. It is not characteristic of any of the protozoan infections. In general, tissue parasites provoke a higher eosinophilia than do parasites that live only in the lumen of the bowel. Significant eosinophilia (20% to 70% or higher) is most frequently seen in trichinosis, strongyloidiasis, hookworm infection, fi lariasis, schistosomiasis, and fasciolopsiasis. Moderate eosinophilia (6% to 20%) is related to trichuriasis, ascariasis, paragonimiasis, taeniasis, and eosinophilic meningitis. **(8)**

1.2.4.4.2: Eosinopenia:

This is a rare, stress-related condition that may be caused by several factors. Eosinopenia is frequently related to the action of glucocorticosteroid hormones or occurs as an aftermath of acute bacterial or viral inflammation.(2)

1.2.4.5: Disorders of Basophils:

1.2.4.5.1: Basophilia:

The number of circulating basophils is not remarkably affected by factors such as time of day, age, and physical activity. Basophilia is considered to exist when the number of basophils exceeds 0.075×109/L. Hormones can cause an increase in basophils, and basophilia can be seen in many disorders, including ulcerative colitis, hyperlipidemia, smallpox, chickenpox, chronic sinusitis, chronic myelogenous leukemia, and polycythemia vera. (2)

1.2.4.5.2: Basopenia:

This condition may be caused by hormones, such as corticotrophin and progesterone, or it may occur at the time of ovulation. Patients with thyrotoxicosis may also have basopenia. (2)

1.2.5: Leukemias:

Leukemia (American English) or leukaemia (British English) is a type of cancer of the blood or bone marrow characterized by an abnormal increase of immature white blood cells called "blasts". Leukemia is a broad term covering a spectrum of diseases. In turn, it is part of the even broader group of diseases affecting the blood, bone marrow, and lymphoid system, which are all known as hematological neoplasms.(8) Leukemia is a treatable disease. Most treatments involve chemotherapy, medical radiation therapy, hormone treatments, or bone marrow transplant. The rate of cure depends on the type of leukemia as well as the age of the patient. Children are more likely to be permanently cured than

adults. Even when a complete cure is unlikely, most people with a chronic leukemia and many people with an acute leukemia can be successfully treated for years. Sometimes, leukemia is the effect of another cancer, known as blastic leukemia, which usually involves the same treatment, although usually unsuccessful.Leukemia can affect people at any age. In 2000 approximately 256,000 children and adults around the world had developed some form of leukemia, and 209,000 have died from it. About 90% of all leukemias are diagnosed in adults. **(8)**

1.2.5.1: Causes and Risk Factors:

There is no single known cause for any of the different types of leukemia. The few known causes, which are not generally factors within the control of the average person, account for relatively few cases. The cause for most cases of leukemia is unknown. The different leukemias likely have different causes. Leukemia, like other cancers, results from mutations in the DNA. Certain mutations can trigger leukemia by activating oncogenes or deactivating tumor suppressor genes, and thereby disrupting the regulation of cell death, differentiation or division. These mutations may occur spontaneously or as a result of exposure to radiation or carcinogenic substances. **(8)** Among adults the known causes are natural and artificial ionizing radiation, a few viruses such as human T-lymphotropic virus, and some chemicals, notably benzene and alkylating chemotherapy agents for previous malignancies. Use of tobacco is associated with a small increase in the risk of developing acute myeloid leukemia in adults. Cohort and case-control studies have linked exposure to some petrochemicals and hair dyes to the development of some forms of leukemia. Diet has very limited or no effect, although eating more vegetables may confer a small protective benefit. Viruses have also been linked to some forms of leukemia. Experiments on mice and other mammals have demonstrated the relevance of retroviruses in leukemia,

and human retroviruses have also been identified. The first human retrovirus identified was human T-lymphotropic virus, or HTLV-1, which is known to cause adult T-cell leukemia. Some people have a genetic predisposition towards developing leukemia. This predisposition is demonstrated by family histories and twin studies. The affected people may have a single gene or multiple genes in common. In some cases, families tend to develop the same kinds of leukemia as other members; in other families, affected people may develop different forms of leukemia or related blood cancers. In addition to these genetic issues, people with chromosomal abnormalities or certain other genetic conditions have a greater risk of leukemia. For example, people with Down syndrome have a significantly increased risk of developing forms of acute leukemia (especially acute myeloid leukemia), and Fanconi anemia is a risk factor for developing acute myeloid leukemia. **(8)**

Whether non-ionizing radiation causes leukemia has been studied for several decades. The International Agency for Research on Cancer expert working group undertook a detailed review of all data on static and extremely low frequency electromagnetic energy, which occurs naturally and in association with the generation, transmission, and use of electrical power. They concluded that there is limited evidence that high levels of ELF magnetic (but not electric) fields might cause childhood leukemia. Exposure to significant ELF magnetic fields might result in twofold excess risk for leukemia for children exposed to these high levels of magnetic fields. However, the report also says that methodological weaknesses and biases in these studies have likely caused the risk to be overstated. No evidence for a relationship to leukemia or another form of malignancy in adults has been demonstrated. Since exposure to such levels of ELFs is relatively uncommon, the World Health Organization concludes that ELF exposure, if later proven to be causative, would

account for just 100 to 2400 cases worldwide each year, representing 0.2 to 4.9% of the total incidence of childhood leukemia for that year (about 0.03 to 0.9% of all leukemias). A few cases of maternal-fetal transmission (a baby acquires leukemia because its mother had leukemia during the pregnancy) have been reported. According to a study conducted at the Center for Research in Epidemiology and Population Health in France, children born to mothers who use fertility drugs to induce ovulation are more than twice more likely to develop leukemia during their childhoods than other children. **(8)**

1.2.5.2: Classification:

Clinically and pathologically, leukemia is subdivided into a variety of large groups. The first division is between its acute and chronic forms:

- Acute leukemia is characterized by a rapid increase in the number of immature blood cells. Crowding due to such cells makes the bone marrow unable to produce healthy blood cells. Immediate treatment is required in acute leukemia due to the rapid progression and accumulation of the malignant cells, which then spill over into the bloodstream and spread to other organs of the body. Acute forms of leukemia are the most common forms of leukemia in children.

- Chronic leukemia is characterized by the excessive build up of relatively mature, but still abnormal, white blood cells. Typically taking months or years to progress, the cells are produced at a much higher rate than normal, resulting in many abnormal white blood cells. Whereas acute leukemia must be treated immediately, chronic forms are sometimes monitored for some time before treatment to ensure maximum effectiveness of therapy. Chronic leukemia mostly occurs in older people, but can theoretically occur in any age group. **(8)**

Additionally, the diseases are subdivided according to which kind of blood cell is affected. This split divides leukemias into lymphoblastic or lymphocytic leukemias and myeloid or myelogenous leukemias:

1. In lymphoblastic or lymphocytic leukemias, the cancerous change takes place in a type of marrow cell that normally goes on to form lymphocytes, which are infection-fighting immune system cells. Most lymphocytic leukemias involve a specific subtype of lymphocyte, the B cell.

2. In myeloid or myelogenous leukemias, the cancerous change takes place in a type of marrow cell that normally goes on to form red blood cells, some other types of white cells, and platelets.

Combining these two classifications provides a total of four main categories. Within each of these four main categories, there are typically several subcategories. Finally, some rarer types are usually considered to be outside of this classification scheme. **(8)**

- Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in young children. This disease also affects adults, especially those ages 65 and older. Standard treatments involve chemotherapy and radiotherapy. The survival rates vary by age: 85% in children and 50% in adults. Subtypes include precursor B acute lymphoblastic leukemia, precursor T acute lymphoblastic leukemia, Burkitt's leukemia, and acute biphenotypic leukemia.

- Chronic lymphocytic leukemia (CLL) most often affects adults over the age of 55. It sometimes occurs in younger adults, but it almost never affects children. Two-thirds of affected people are men. The five-year survival rate is 75%. It is incurable, but there are many effective treatments. One subtype is B-cell prolymphocytic leukemia, a more aggressive disease. **(8)**

- Acute myelogenous leukemia (AML) occurs more commonly in adults than in children, and more commonly in men than women. AML is treated with chemotherapy. The five-year survival rate is 40%, except for APL, which is over 90%. Subtypes of AML include acute promyelocytic leukemia, acute myeloblastic leukemia, and acute megakaryoblastic leukemia.

- Chronic myelogenous leukemia (CML) occurs mainly in adults; a very small number of children also develop this disease. Treatment is with imatinib (Gleevec in United States, Glivec in Europe) or other drugs. The five-year survival rate is 90%. One subtype is chronic myelomonocytic leukemia.

- Hairy cell leukemia (HCL) is sometimes considered a subset of chronic lymphocytic leukemia, but does not fit neatly into this pattern. About 80% of affected people are adult men. No cases in children have been reported. HCL is incurable, but easily treatable. Survival is 96% to 100% at ten years.

- T-cell prolymphocytic leukemia (T-PLL) is a very rare and aggressive leukemia affecting adults; somewhat more men than women are diagnosed with this disease. Despite its overall rarity, it is also the most common type of mature T cell leukemia; nearly all other leukemias involve B cells. It is difficult to treat, and the median survival is measured in months. **(8)**

- Large granular lymphocytic leukemia may involve either T-cells or NK cells; like hairy cell leukemia, which involves solely B cells, it is a rare and indolent (not aggressive) leukemia.

- Adult T-cell leukemia is caused by human T-lymphotropic virus (HTLV), a virus similar to HIV. Like HIV, HTLV infects CD4+ T-cells and replicates within them; however, unlike HIV, it does not destroy them. Instead, HTLV "immortalizes" the infected T-cells,

giving them the ability to proliferate abnormally. Human T cell lymphotropic virus types I and II (HTLV-I/II) are endemic in certain areas of the world.

1.2.5.3: Symptoms of Leukemia:

Damage to the bone marrow, by way of displacing the normal bone marrow cells with higher numbers of immature white blood cells, results in a lack of blood platelets, which are important in the blood clotting process. This means people with leukemia may easily become bruised, bleed excessively, or develop pinprick bleeds (petechiae). White blood cells, which are involved in fighting pathogens, may be suppressed or dysfunctional. This could cause the patient's immune system to be unable to fight off a simple infection or to start attacking other body cells. Because leukemia prevents the immune system from working normally, some patients experience frequent infection, ranging from infected tonsils, sores in the mouth, or diarrhea to life-threatening pneumonia or opportunistic infections. Finally, the red blood cell deficiency leads to anemia, which may cause dyspnea and pallor.Some patients experience other symptoms, such as feeling sick, having fevers, chills, night sweats, feeling fatigued and other flu-like symptoms. Some patients experience nausea or a feeling of fullness due to an enlarged liver and spleen; this can result in unintentional weight loss. Blasts affected by the disease may come together and become swollen in the liver or in the lymph nodes causing pain and leading to nausea. If the leukemic cells invade the central nervous system, then neurological symptoms (notably headaches) can occur. Uncommon neurological symptoms like migraines, seizures, or coma can occur as a result of brain stem pressure. All symptoms associated with leukemia can be attributed to other diseases. Consequently, leukemia is always diagnosed through medical tests. The word leukemia, which means 'white blood', is derived from the disease's

namesake high white blood cell counts that most leukemia patients have before treatment. The high number of white blood cells is apparent when a blood sample is viewed under a microscope. Frequently, these extra white blood cells are immature or dysfunctional. The excessive number of cells can also interfere with the level of other cells, causing a harmful imbalance in the blood count. Some leukemia patients do not have high white blood cell counts visible during a regular blood count. This less-common condition is called aleukemia. The bone marrow still contains cancerous white blood cells which disrupt the normal production of blood cells, but they remain in the marrow instead of entering the bloodstream, where they would be visible in a blood test. For an aleukemic patient, the white blood cell counts in the bloodstream can be normal or low. Aleukemia can occur in any of the four major types of leukemia, and is particularly common in hairy cell leukemia. **(8)**

1.2.5.4: Diagnosis:

Diagnosis is usually based on repeated complete blood counts and a bone marrow examination following observations of the symptoms, however, in rare cases blood tests may not show if a patient has leukemia, usually this is because the leukemia is in the early stages or has entered remission. A lymph node biopsy can be performed as well in order to diagnose certain types of leukemia in certain situations. Following diagnosis, blood chemistry tests can be used to determine the degree of liver and kidney damage or the effects of chemotherapy on the patient. When concerns arise about visible damage due to leukemia, doctors may use an X-ray, MRI, or ultrasound. These can potentially view leukemia's effects on such body parts as bones (X-ray), the brain (MRI), or the kidneys, spleen, and liver (ultrasound). Finally, CT scans are rarely used to check lymph nodes in the chest.

Despite the use of these methods to diagnose whether or not a patient has leukemia, many people have not been diagnosed because many of the symptoms are vague, unspecific, and can refer to other diseases. For this reason, the American Cancer Society predicts that at least one-fifth of the people with leukemia have not yet been diagnosed. Mutation in SPRED1 gene has been associated with a predisposition to childhood leukemia.SPRED1 gene mutations can be diagnosed with genetic sequencing. **(8)**

1.2.5.5: Types of Leukemias:

1.2.5.5.1: Acute lymphoblastic Leukemia:

Management of ALL focuses on control of bone marrow and systemic (whole-body) disease. Additionally, treatment must prevent leukemic cells from spreading to other sites, particularly the central nervous system (CNS) e.g. monthly lumbar punctures. In general, ALL treatment is divided into several phases:

- Induction chemotherapy to bring about bone marrow remission. For adults, standard induction plans include prednisone, vincristine, and an anthracycline drug; other drug plans may include L-asparaginase or cyclophosphamide. For children with low-risk ALL, standard therapy usually consists of three drugs (prednisone, L-asparaginase, and vincristine) for the first month of treatment.

- Consolidation therapy or intensification therapy to eliminate any remaining leukemia cells. There are many different approaches to consolidation, but it is typically a high-dose, multi-drug treatment that is undertaken for a few months. Patients with low- to average-risk ALL receive therapy with antimetabolite drugs such as methotrexate and 6-mercaptopurine (6-MP). High-risk patients receive higher drug doses of these drugs, plus additional drugs.

- CNS prophylaxis (preventive therapy) to stop the cancer from spreading to the brain and nervous system in high-risk patients. Standard prophylaxis may include radiation of the head and/or drugs delivered directly into the spine.

- Maintenance treatments with chemotherapeutic drugs to prevent disease recurrence once remission has been achieved. Maintenance therapy usually involves lower drug doses, and may continue for up to three years.

- Alternatively, allogeneic bone marrow transplantation may be appropriate for high-risk or relapsed patients. **(8)**

1.2.5.5.2: Chronic lymphocytic leukemia:

Hematologists base CLL treatment on both the stage and symptoms of the individual patient. A large group of CLL patients have low-grade disease, which does not benefit from treatment. Individuals with CLL-related complications or more advanced disease often benefit from treatment. In general, the indications for treatment are:

- Falling hemoglobin or platelet count
- Progression to a later stage of disease
- Painful, disease-related overgrowth of lymph nodes or spleen
- An increase in the rate of lymphocyte production

1.2.5.5.3: Acute myelogenous leukemia:

Many different anti-cancer drugs are effective for the treatment of AML. Treatments vary somewhat according to the age of the patient and according to the specific subtype of AML. Overall, the strategy is to control bone marrow and systemic (whole-body) disease, while offering specific treatment for the central nervous system (CNS), if involved.

In general, most oncologists rely on combinations of drugs for the initial, induction phase of chemotherapy. Such combination chemotherapy usually offers the benefits of early remission and a lower risk of disease resistance. Consolidation and maintenance treatments are intended to prevent disease recurrence.Consolidation treatment often entails a repetition of induction chemotherapy or the intensification chemotherapy with additional drugs. By contrast, maintenance treatment involves drug doses that are lower than those administered during the induction phase. **(8)**

1.2.5.5.4: Chronic myelogenous leukemia:

There are many possible treatments for CML, but the standard of care for newly diagnosed patients is imatinib (Gleevec) therapy. Compared to most anti-cancer drugs, it has relatively few side effects and can be taken orally at home. With this drug, more than 90% of patients will be able to keep the disease in check for at least five years, so that CML becomes a chronic, manageable condition.In a more advanced, uncontrolled state, when the patient cannot tolerate imatinib, or if the patient wishes to attempt a permanent cure, then an allogeneic bone marrow transplantation may be performed. This procedure involves high-dose chemotherapy and radiation followed by infusion of bone marrow from a compatible donor. Approximately 30% of patients die from this procedure.

1.2.5.5.5: Hairy cell leukemia:

Hairy cell leukemia is an uncommon form of chronic leukemia characterized by circulating cells with filamentous cytoplasmic projections (hairy cells), neutropenia, splenomegaly, and fibrosis in the bone marrow. Hairy cell leukemia appears in the bone marrow as a proliferation of cells with round, oval, or slightly irregular nuclei. The cells have abundant cytoplasm, which appears clear. The nuclei are widely spaced and appear surrounded by a clear space, giving a "fried

egg" appearance. The pattern of infiltration is usually interstitial or diffuse. Fibrosis is common, resulting in frequent inaspirable marrow (dry taps). **(7)**

1.2.5.5.6: T-cell prolymphocytic leukemia:

Most patients with T-cell prolymphocytic leukemia, a rare and aggressive leukemia with a median survival of less than one year, require immediate treatment. T-cell prolymphocytic leukemia is difficult to treat, and it does not respond to most available chemotherapeutic drugs.[34] Many different treatments have been attempted, with limited success in certain patients: purine analogues (pentostatin, fludarabine, cladribine), chlorambucil, and various forms of combination chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisone CHOP, cyclophosphamide, vincristine, prednisone [COP], vincristine, doxorubicin, prednisone, etoposide, cyclophosphamide, bleomycin VAPEC-B). Alemtuzumab (Campath), a monoclonal antibody that attacks white blood cells, has been used in treatment with greater success than previous options. Some patients who successfully respond to treatment also undergo stem cell transplantation to consolidate the response. **(8)**

1.2.5.5.7: Juvenile myelomonocytic leukemia:

Treatment for juvenile myelomonocytic leukemia can include splenectomy, chemotherapy, and bone marrow transplantation. **(8)**

1.3: Objectives:

1.3.1: General objective:

To estimate the normal values for Sudanese white blood cells count for the work in public and private hospitals.

1.3.2: Specific objectives:

- To count TWBCs, differential count and absolute values.

- To compare hematological parameters with the universal normal values of these parameters.

- To determine the local reference values for these ages.

2.1: Study area:

Blood samples were collected from Sudan University and Zhat alnetakeen basic school.

2.2: Study population:

This study done on blood samples collected from healthy Sudanese individuals by excluding allergy, infections and drug use.

2.3: Study location:

The study was performed in Sudan University of medical laboratory science.

2.4: Study duration:

The study was conducted during the period from February till March 2014.

2.5: Sample size:

The total sample size was 200 samples.

2.6: Statistical analysis:

Statistical analysis done using statistical package for the social sciences (SPSS) computerized programme.

2.7: study design:

This is analytical descriptive case study.

2.8: Inclusion criteria:

Must be collected from healthy individuals.

2.9: Exclusion criteria:

Inflamed, infected, burned, allergic patients were excluded.

2.10: sampling and sample size:

200 Ethylene Di amine Tetra Acetic Acid (EDTA) venous blood collected from healthy individuals was conducted during the period from February till March 2014.

2.11: Ethical consideration:

- Study population was provided with enough knowledge about study and its values.

- The agreement was taken from population without compulsion.

2.12: Subject:

Blood sample collected from 200 healthy individuals in February 2014 to mars 2014 in Khartoum state. The CBC performed to evaluate the TWBCs and differential count and the thin blood film to show the morphology.

2.13: Materials (Equipment and Reagent):

- Cotton.

- Sterile syringe.

- 75% ethyl or methyl alcohol.

- Tourniquet.

- Plastic containers (EDTA).

- Clean slides.

- Marker (Slide labeling).

- Leishman Stain.

- Chamber (Hemocytometer).

- 2% acetic acid.

- 5mL pipette.

- 0.02 ml pipette.

- Pastier pipette.

- Test tubes.

- Cover glasses.

- Differential tally counter.

- Microscope.

2.14: Method:

2.14.1: Blood Collection:

Clean the venous puncture site. With a sterile disposable syringe, collect the blood then transfer it to container. In the meantime, keep all the materials needed ready and protected from dust, particularly the clean microscope slides.

2.14.2: Analyzing by manual method (Total WBCs Count):

Blood sample is mixed and diluted with a weak concentration of

Hydrochloric acid (HCL), or acetic acid (in specified known volumes). Weak Acids will lyse red blood cells, and will darken WBC's to facilitate counting by the chamber. The use of hemocytometer counting chamber for manually counting WBCs and platelets. **(1)**

2.14.2.1:The steps are:

1- Mix the blood sample gently but thoroughly by inversion, manually or by mechanical rocking mixer.

2- Pipette 0.38 ml (380 ml) of diluting fluid into a 12x75 mm tube.

3- Pipette 0.02 ml (20 ml) of well mixed blood to be counted and wipe the tip with gauze into the tube containing diluting fluid and mix the tube.

4- Let the tube stand for 2-3 minutes to ensure complete RBC lyses, then mix well.

5- Prepare the clean chamber and cover it with the cover slip.

6- Load one side of the chamber with the aid of a capillary tube or Micropipette, do not attempt to overload or under load the chamber .

7- Allow the chamber to sit for several minutes to allow the WBC's to settle in the counting chamber, to avoid drying effect, place the loaded chamber in a covered Petri dish with moist gauze, until counting.

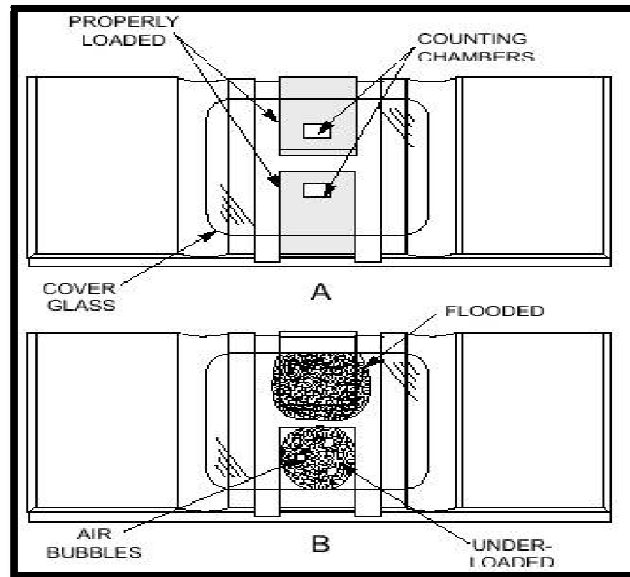8- Place the chamber in the microscope stage using ×10 objective.
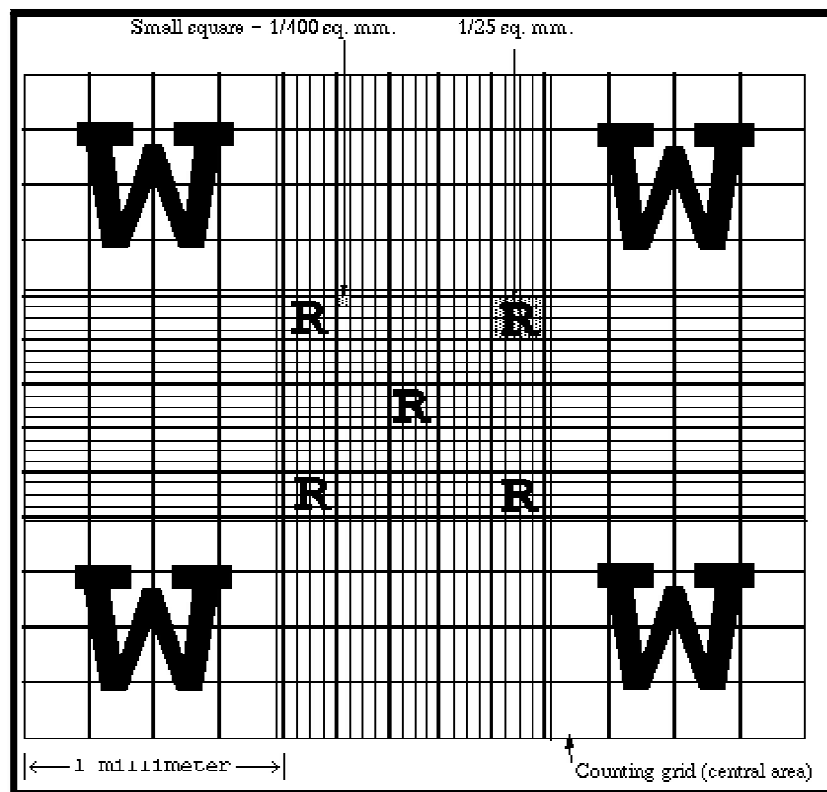
Figure (2.1): Loading the chamber

Figure (2.2): The counting area of the chamber**.**

Calculation:

Count (per liter) =

Sum ×dilution factor (20) / depth (0.1)×area count (4) = Sum×50

2.14.3: Making of Smear:

Place a small drop of blood near the end of a slide. Bring the edge of another slide in contact with the drop and allow the drop to bank evenly behind the spreader. The angle between the two slides has to be 30-40 degrees. Now, push to the left in a smooth, quick motion. The smear should cover half of the slide. It is important that the quantity of the blood is not excessive; otherwise the red cells could hide the leukocytes. So, if you succeed in making a gradual transition from thick to thin in your smear, you should get a zone with a satisfactory distribution of cells.With a single drop of blood, you can make several smears. In fact, to make a smear it is enough to leave a spot of blood of 3mm about 1 diameter on the slide. It is useful to perform many smears. To avoid producing clots, you must make smear with fresh blood and straight after having deposited it, for this purpose, it is useful to be helped by another person where one deposits the blood, and the other makes the smear. With the microscope you should observe the smear to check that some of them are properly made. The red cells must not overlap each other, nor be so scarce as to be too spread out.

2.14.3.1: Staining (Leishman's stain):

2.14.3.1.1: Leishman's stain preparation:

Weigh out 0.2 g of the powdered dye, and transfer it to a conical flask of 200–250 ml capacity. Add 100 ml of methanol and warm the mixture to 50_C for 15 min, occasionally shaking it. Allow the flask to cool and filter the solution. It is then ready for use, but it will improve on standing.[1]

2.14.3.1.2: Leishman's Stain method:

Air dry the film and flood the slide with the stain. After 2 min, add double the volume of water and stain the film for 5–7 min. Then wash it in a stream of buffered water until it has acquired a pinkish tinge (up to 2 min). After the back of the slide has been wiped clean, set it up right to dry. **(1)**

2.14.3.2: Checking:

With the microscope, verify that the cells are well stained. If necessary, apply the stain for a few minutes. If you were planning to mount the slide with Canada balsam, the staining has to be stronger.

2.14.4: Differential leukocyte count:

Differential leukocyte counts are usually performed by visual examination of blood films that are prepared on slides by the spread or 'wedge' technique. **(1)**

Manual differentials are performed by taking a drop of blood, spreading it on a slide, staining it, and evaluating **100** cells individually for quality and changes in morphology and count by using differential tally counter.

1- Focus the film under x10 lens, and scan the film to check cell distribution.

2- Add a drop of oil, and move to the x100 oil immersion lens.

3- Choose a suitable area, where cells are evenly distributed without appreciable overlapping- the monolayer cell zone.

4- Count the WBC's using tracking pattern.

5- Each cell identified should be immediately tallied as:

· Neutrophil- segmented.

· Neutrophil – band.

· Lymphocyte.

· Monocyte.

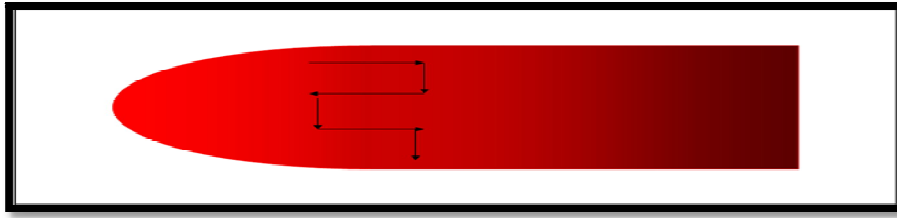· Eosinophil.

· Basophil.

Figure (2.3): Differential counting from the thin film

2.14.5: Absolute leukocyte count:

It's done to differentiate between real and relative increase or decrease of special type of cells.

Absolute= Differential count/100 × TWBCs

3.1:**The Result:**

Table(3.1): Whole Results of Research:

Parameter	Mean	Std deviation
TWBCs	5.9	1.17
Neutrophil%	55.3	6.99
Lymphocyte%	35.5	6.50
Monocyte%	6.2	2.03
Eosinophil%	2.8	1.42
Basophil%	0.17	0.37
Neutrophil Absolute Count	3.3	1.24
Lymphocyte Absolute Count	2.0	0.65
Monocyte Absolute Count	0.36	0.13
Eosinophil Absolute Count	0.15	0.08
Basophil Absolute Count	0.00	0.02

Table (3.2): Showing the Age of the samples & comparing their means TWBCs

Age	Percent%	Mean
10-15 year	25%	5.9
16- 30 year	25%	5.2
31-50year	26%	6.5
51-65 year	24%	6.2
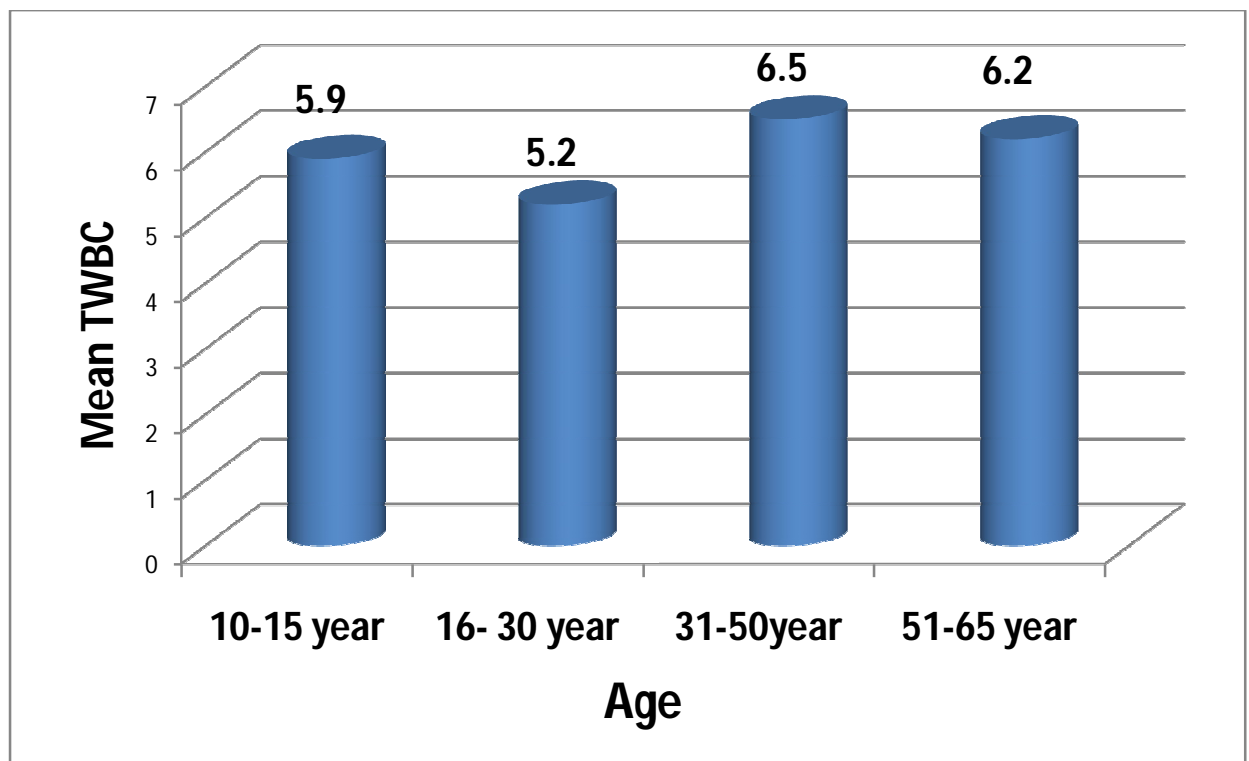
Figure (3.1): Mean of TWBCs for Age Groups

Table (3.3): Showing the Age of the samples & comparing their means Neutrophil%

Age	Percent%	Mean
10-15 year	25%	55.5
16- 30 year	25%	53.9
31-50year	26%	55.1
51-65 year	24%	56.7
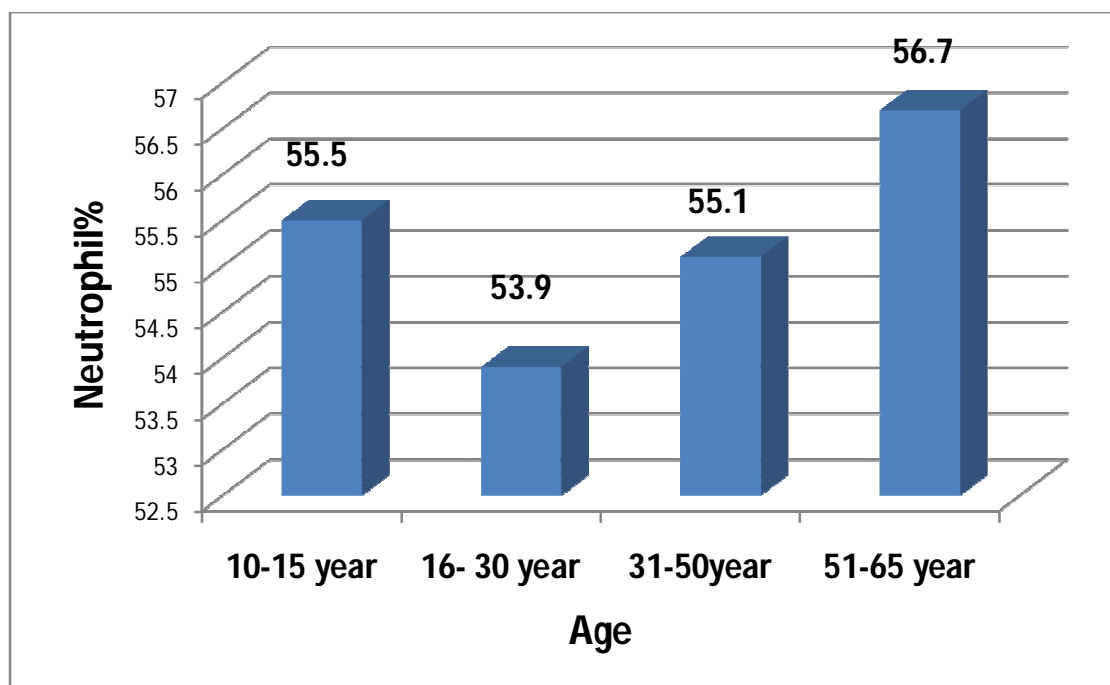
Figure (3.2): Mean of Neutrophil% for Age Groups

Table (3.4): Showing the Age of the samples & comparing their means Lymphocyte%

Age	Percent%	Mean
10-15 year	25%	35.1
16- 30 year	25%	35.5
31-50year	26%	36.4
51-65 year	24%	35.2
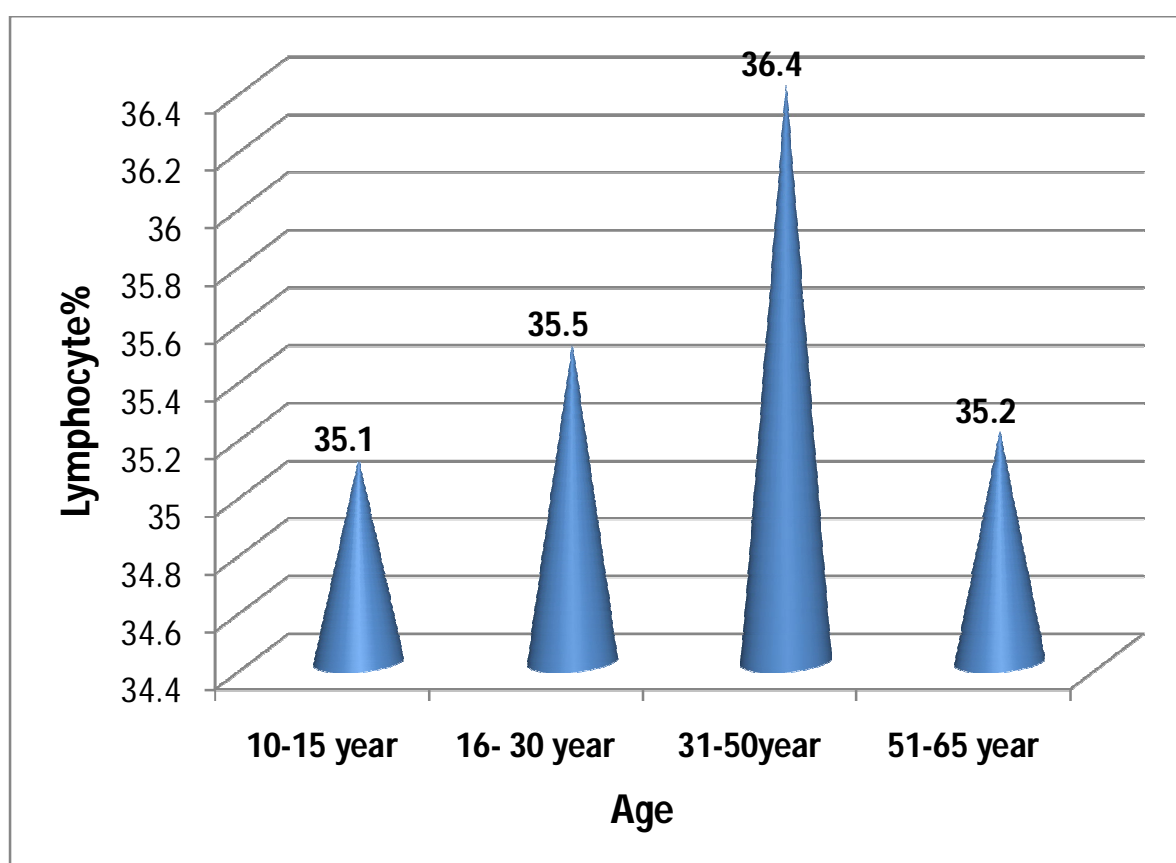
Figure (3.3): Mean of Lymphocyte% for Age Groups

Table (3.5): Showing the Age of the samples & comparing their means
Monocyte %

Age	Percent%	Mean
10-15 year	25%	6.9
16- 30 year	25%	6.8
31-50year	26%	6.1
51-65 year	24%	6.3
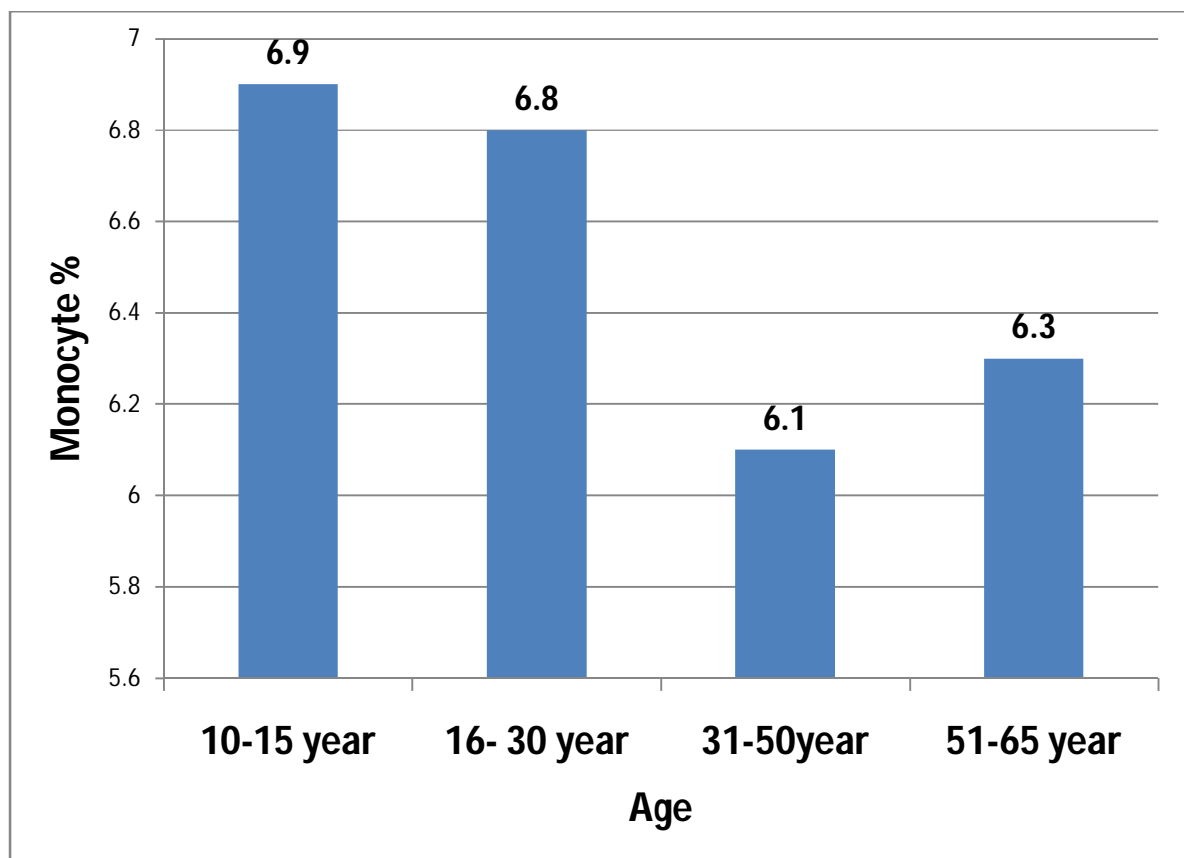
Figure (3.4): Mean of Monocyte% for Age Groups

Table (3.6): Showing the Age of the samples & comparing their means Eosinophil%

Age	Percent%	Mean
10-15 year	25%	2.7
16- 30 year	25%	2.9
31-50year	26%	2.6
51-65 year	24%	2.8
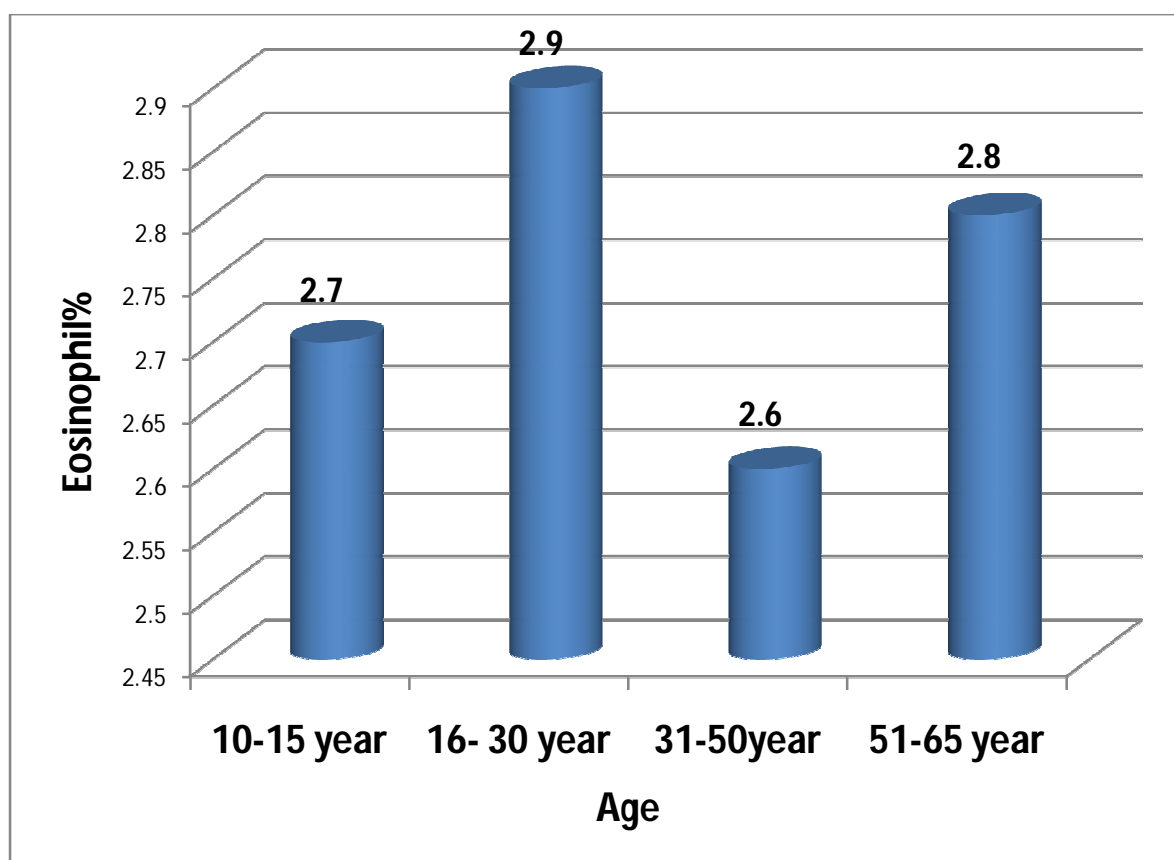
Figure (3.5): Mean of Eosinophil% for Age Groups

Table (3.7): Showing the Age of the samples & comparing their means Basophil%

Age	Percent%	Mean
10-15 year	25%	.12
16- 30 year	25%	.28
31-50year	26%	.13
51-65 year	24%	.21
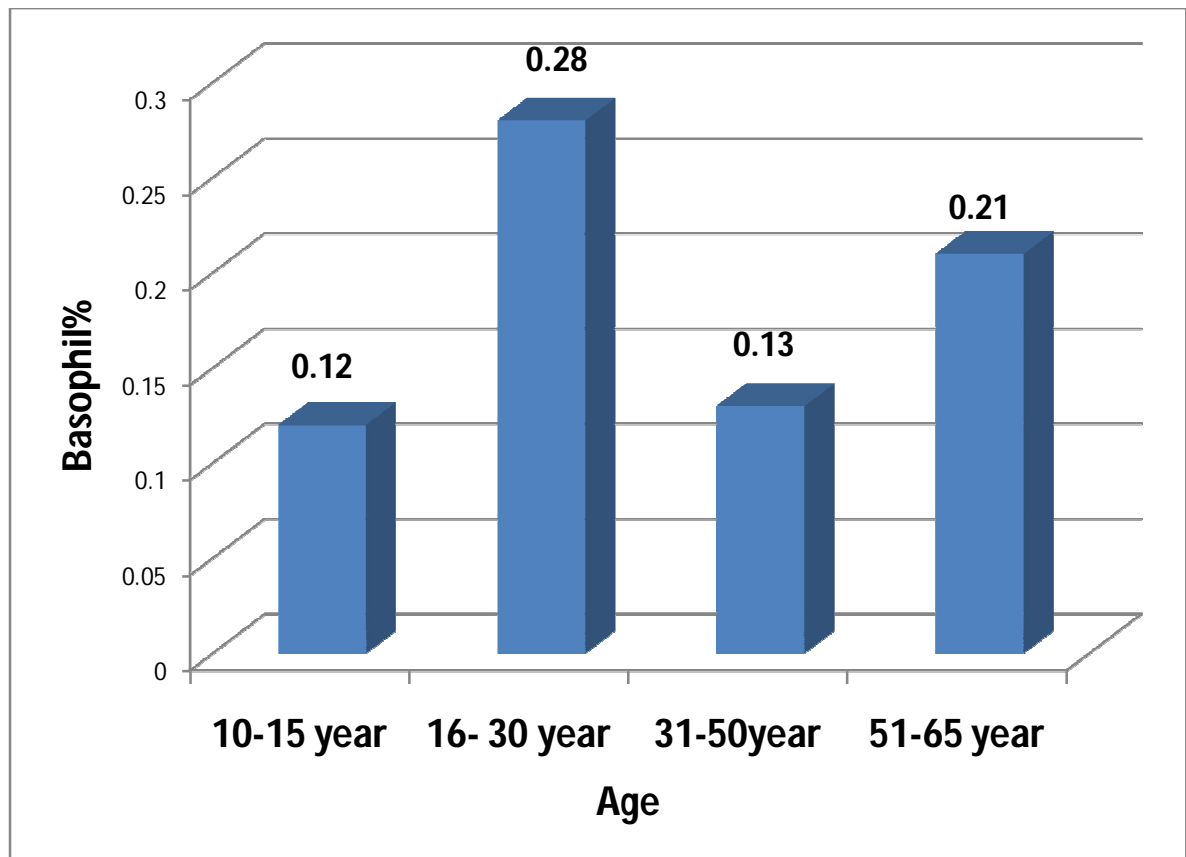
Figure (3.6): Mean of Basophil % for Age Groups

Table (3.8): Showing the gender of the samples & comparing their means TWBCs
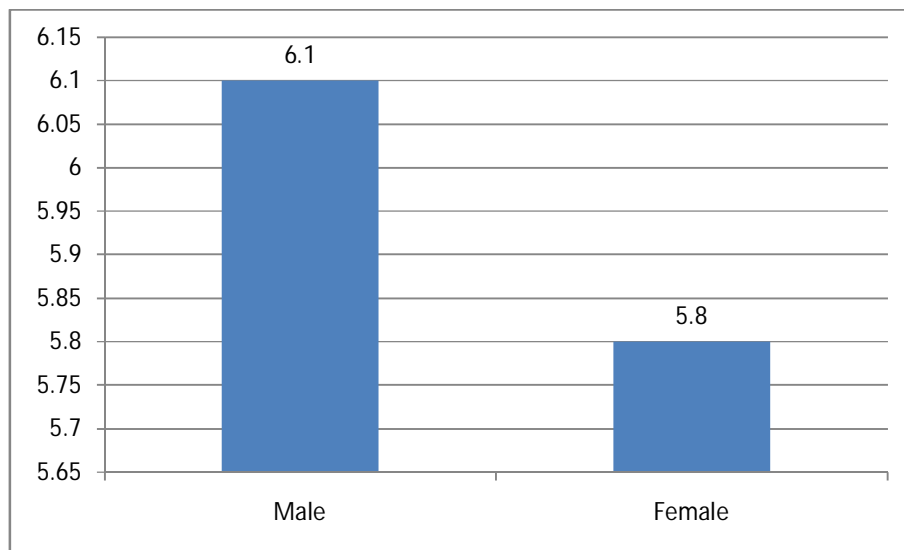
Gender	Percent%	Mean
Male	46%	6.1
Female	54%	5.8

Figure (3.7): Mean of TWBCs for Male & Female

Table (3.9): Showing the gender of the samples & comparing their means Neutrophil%

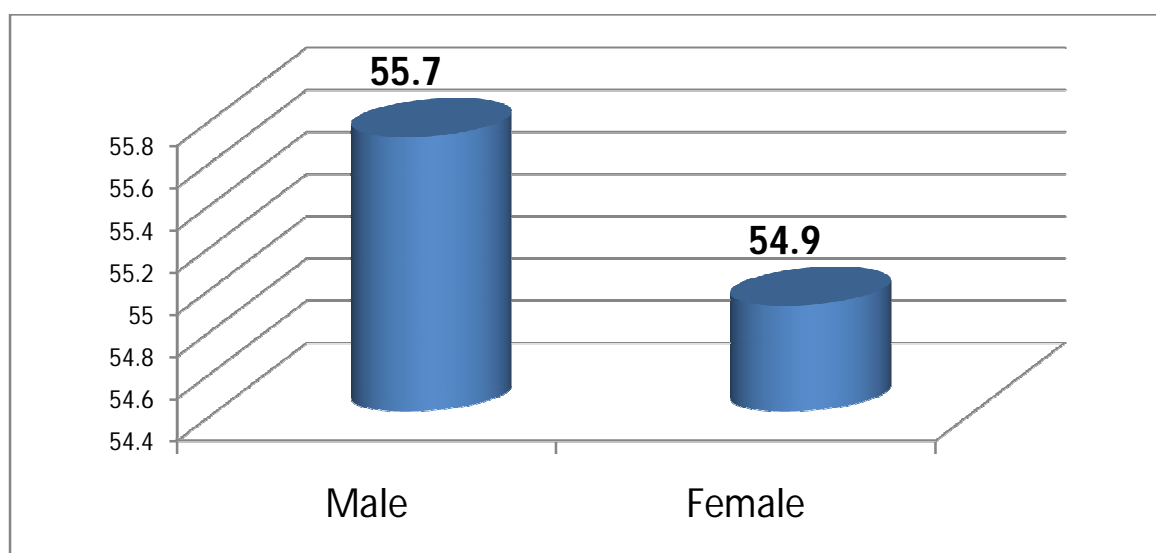| Gender	Percent%	Mean
Male	46%	55.7
Female	54%	54.9

Figure (3.8): Mean of Neutrophil% for Male & Female

Table (3.10): Showing the gender of the samples & comparing their means Lymphocyte%

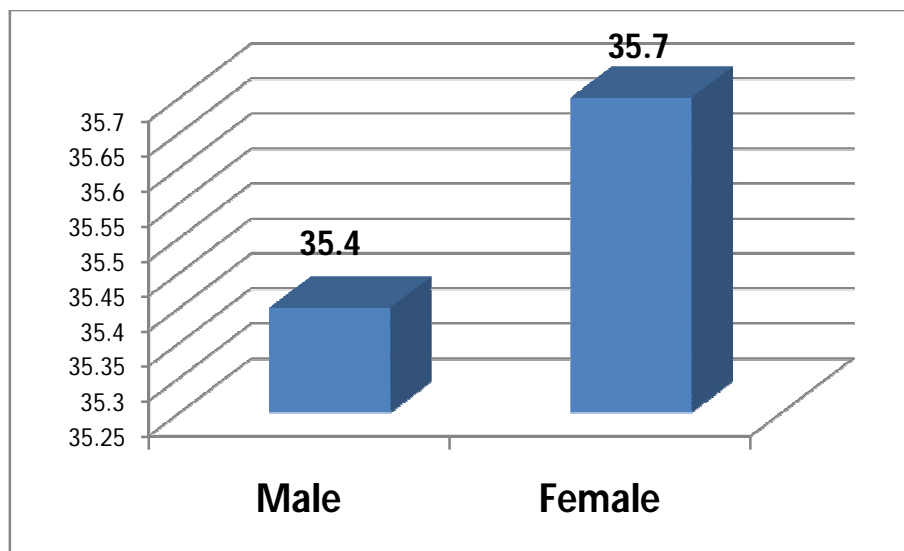| Gender	Percent%	Mean
Male	46%	35.4
Female	54%	35.7

Figure (3.9): Mean of Lymphocyte% for Male & Female

Table (3.11): Showing the gender of the samples & comparing their means Monocyte %

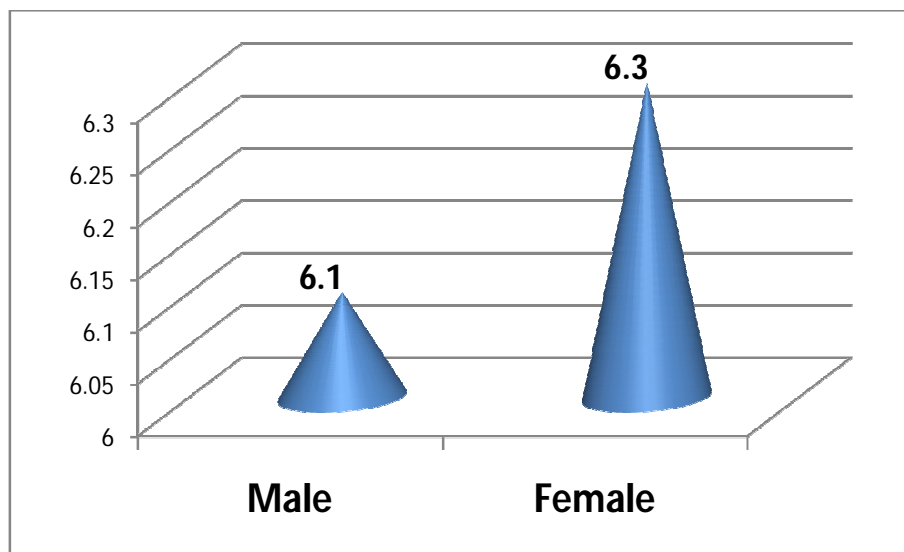| Gender	Percent%	Mean
Male	46%	6.1
Female	54%	6.3

Figure (3.10): Mean of Monocyte %for Male & Female

Table (3.12): Showing the gender of the samples & comparing their means Eosinophil %

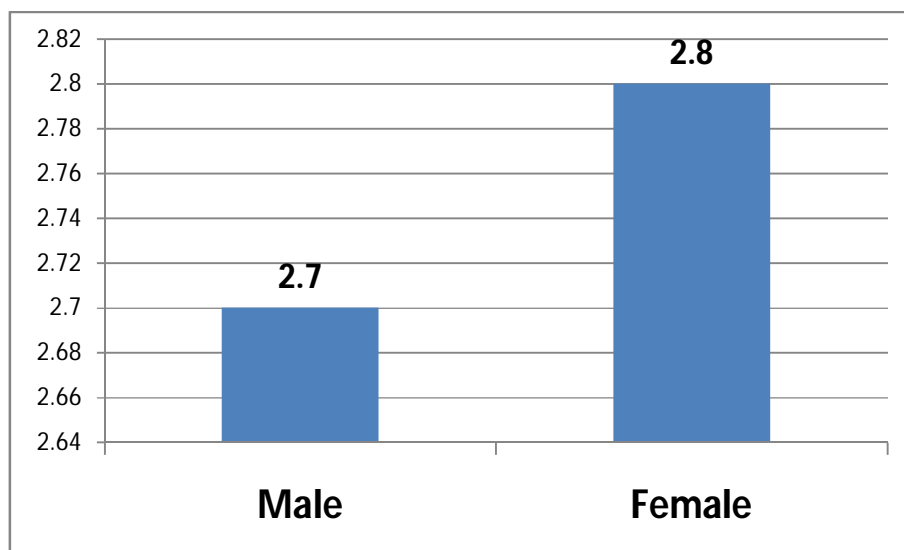| Gender	Percent%	Mean
Male	46%	2.7
Female	54%	2.8

Figure (3.11): Mean of Eosinophil %for Male & Female

Table (3.13): Showing the gender of the samples & comparing their means Basophil %

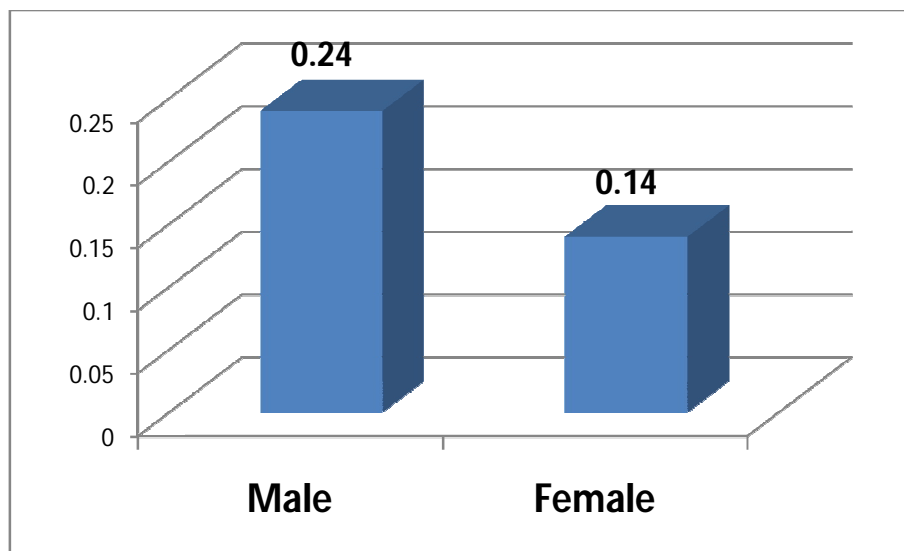| Gender	Percent%	Mean
Male	46%	.24
Female	54%	.14

Figure (3.12): Mean of Basophil % for Male & Female

4.1: Discussion:

This study provides normal values of TWBCs and absolute leukocyte count of healthy individuals who of (10-65) years old in period from 6\2\2014 up to 10\3\2014 in Khartoum residents, there was 92 males and 108 females. The result shown that the mean of TWBCs in male 6.0 ×10^9/L, female 5.8 ×10^9/L, TWBCs mean 5.9 ×10^9/L, the mean of Neutrophil 55.3%, Lymphocyte 35.5%, Monocyte 6.2%, Eosinophil 2.8%, Basophil 0.17%, the mean of absolute count for Neutrophil 3.3 ×10^9/L, Lymphocyte 2.0 ×10^9/L, Monocyte 0.36 ×10^9/L, Eosinophil 0.15 ×10^9/L, Basophil 0.0 ×10^9/L. The normal value of TWBCs 3.3 - 10.7 ×10^9/L, Neutrophil 35-75%, Lymphocyte 20-55%, Monocyte 1-10%, Eosinophil 0-6%, Basophil 0-1%, the normal range of the absolute count for the Neutrophil 1.7-7.7 ×10^9/L, Lymphocyte 0.7- 5.0 ×10^9/L, Monocyte 0.04-0.9 ×10^9/L, Eosinophil 0.0-0.5 ×10^9/L, Basophil 0.0-0.13 ×10^9/L. Old people show decrease in TWBCs count and lymphocyte count, but shown also increase in neutrophil count when compared against individuals at young age. Pervious study done on people living in the same community in Britain but drawn from four different ethnic groups. The groups were white (northern Europeans), Indians, black (African and West Indians) and oriental. Black people showed significant decrease in TWBCs and absolute count which agreed with our research results. White people had higher absolute monocyte count while Indians had high eosinophilic count than whites. No ethnic variation was found in absolute lymphocyte count. They found that the difference between the count of Indians, oriental and those of whites was minor and for practical purposes they can be assessed in relation to reference range derived from whites while blacks shouldn't be assessed by their reference range. Also Caucasians studies on TWBCs count showed that Africans and Afro-carribians had much lower neutrophil

count than Caucasians and the count was lower in Africans than Afro-carribians.

4.2: Conclusion:

Reference value of TWBCs in Sudanese individuals of age (10-65) years old was decreased than the world reference range. The research without is significant and the individuals without is significant and the individuals with TWBCs count higher than 10.7 $\times 10^9$/L must consider as abnormal benign leukocytosis.

4.3: Recommendation:

- The new normal values should be used in Sudanese public and private hospitals.

- Normal value of TWBCs should be established by using the flowcytometer instrument to get more accurate values.

- Reference value of all laboratory parameters should be estimated.

- Reference value of TWBCs count and absolute count of age less than 10 and higher than 65 including neonates should be estimated.

- The different tribes in Sudan should be included in the further study.

- More studies should be done with more sample size.

5.1: References:

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Chamber

Syringe

Slides

EDTA container