Chapter One

Introduction and Literature Review

1.1 Haemopoiesis

The process that leads to the production and regulation of blood cells is called hematopoiesis. It consists of mechanisms triggering differentiation and maturation of hematopoietic stem cells. Located in the bone marrow, hematopoietic stem cells are undifferentiated cells, unobservable directly (even though they can be tracked by markers), with unique capacities of differentiation (the ability to produce cells committed to one of blood cell types) and self-renewal (the ability to produce an identical cell with the same properties). Under the action of growth factors (molecules acting like hormones playing an activator/inhibitor role), hematopoietic stem cells produce differentiated cells throughout cell divisions until blood cells (White cells, red blood cells, and platelets) are formed and ready to enter the bloodstream. Blood is a life-sustaining fluid which circulates through the heart and blood vessels. It carries oxygen and nutrients to the tissues and waste products to the lungs, liver and kidneys, where they can be removed from the body (Barbara, 2004). 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 bone 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 sinus spaces, 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 humeral (Table 1.1). Even in these haemopoietic areas, approximately 50% of the marrow consists of fat (Fig. 1.1). 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 haemopoieticrole ('extramedullary haemopoiesis) (Hoffbrand et. al, 2006).

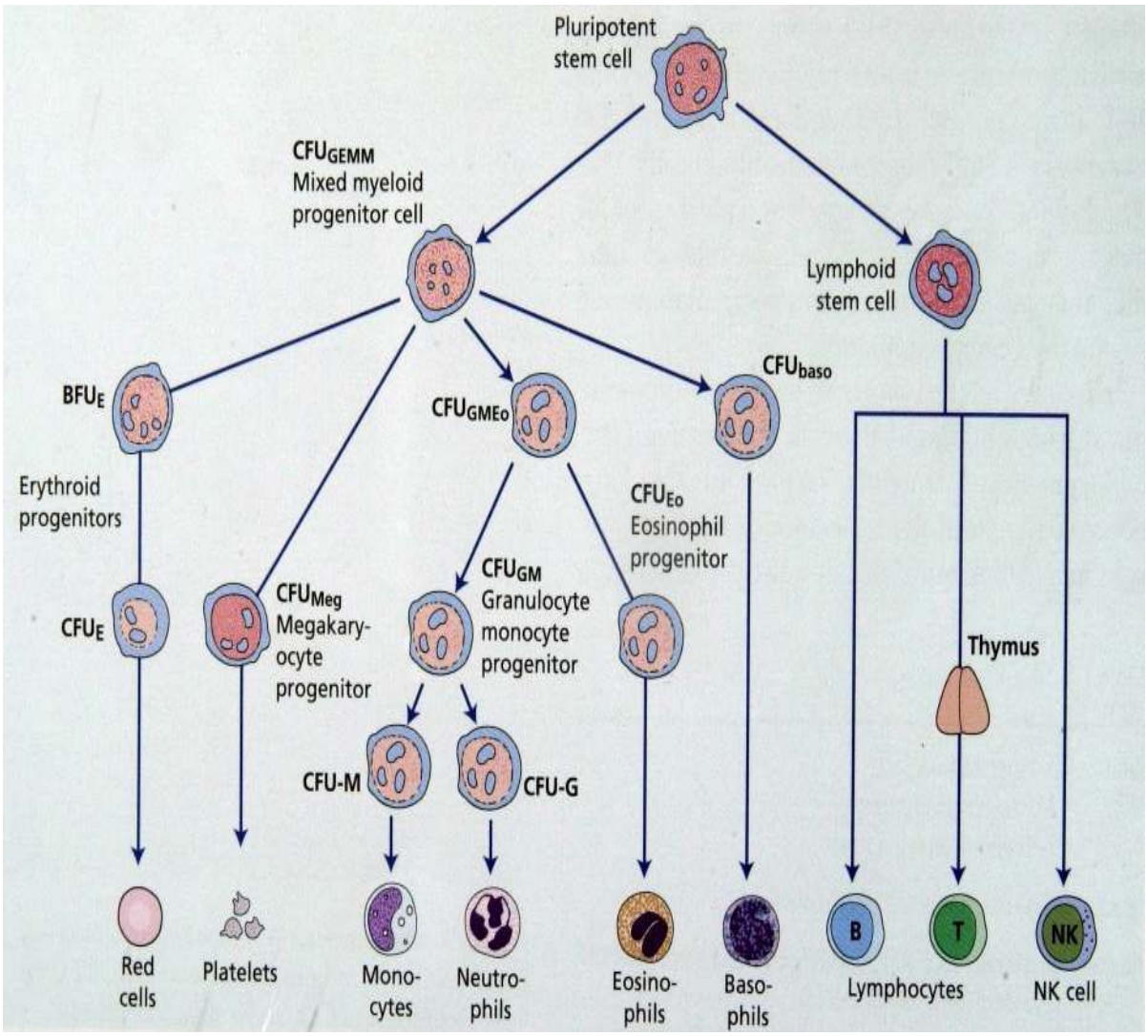
Hematopoietic growth factors are proteins or glycoproteins that regulate the production and differentiation of hematopoietic precursors. They act on specific cell surface receptors on hematopoietic precursor cells and may either stimulate or inhibit cell proliferation and differentiation. A large number of growth

factors have been identified, including various interleukins (produced by lymphocytes), colony-stimulating factors (CSFs), and others. The majority is produced within the marrow and act locally. Erythropoietin and thrombopoietin are produced outside the marrow and reach the marrow through the blood. Growth factors may affect multiple cell lines and act at multiple stages; a few are relatively lineage specific (for example, the effects of erythropoietin are primarily limited to erythroid precursors). There are complex interactions between different growth factors in the differentiation of different cell types. Growth factors may have effects on mature cells, as well as on the proliferation and maturation of hematopoietic precursors. For example, granulocyte colony-stimulating factor (G-CSF) can activate mature neutrophils, and granulocyte-macrophage colony-stimulating factor (GM-CSF) can activate both monocytes and granulocytes) (William, 2002).

Table 1-1 site of hemopoiesis (Hoff brand et. al, 2006).

Fetus	0-2 months (yolk sac) 2-7 months (liver, spleen) 5-9 months(bone marrow)
Infants	Bone marrow (all bones)
Adults	Vertebrae,ribs,sternum,skull, sacrum and pelvis, proximal ends of femur.

Figure 1-1 Haemopoiesis (Hoff brand et. al, 2006).



1.1.1 Erythropoies:

Erythropoies is passes from the stem cell through the progenitor cells colony-forming milt granulocyte, erythroid, monocyte and megakaryocyte (CFU_{CEMM}) burst forming milt erthyroid (BFUE) and erthroid CFU (CFUE) to the first recognizable erythrocyte precursor in the bone marrow, the pro normoblast. This is a large cell with dark blue cytoplasm, a central nucleus with nucleoli and

slightly clumped chromatin .The pro normoblast gives rise to a series of progressively smaller nonnoblasts by a number of cell divisions. They also contain progressively more haemoglobin (which stains pink) in the cytoplasm; the cytoplasm stains paler blue as it loses its RNA and protein synthetic apparatus while nuclear chromatin becomes more condensed. The nucleus is finally extruded the late normoblast within the marrow and a reticulocyte stage results which still contains some ribosomal RNA and is still able to synthesize haemoglobin. This cell is slightly larger than a mature red cell, spends 1-2 days in the marrow and also circulates in the peripheral blood for 1-2 days before maturing, mainly in the spleen, when RNA is completely lost (Hoffbrand et. al, 2006).

Erythropoies is regulated by the hormone erythropoietin. The erythropoietin gene contains a hypoxia response element at its 3' end. Erythropoietin is a heavily glycosylated polypeptide of 165 amino acids with a molecular weight of 34 kDa. Normally, 90% of the hormone is produced in the peritubular interstitial cells of the kidney and 10% in the liver and elsewhere. There are no preformed stores and

the stimulus to erythropoietin production is the oxygen (O2) tension in the tissues of the kidney. Erythropoietin production therefore increases in anaemia, when haemoglobin for some metabolic or structure reason is unable to give up O2 normally, when atmospheric O2 is low or when defective cardiac or

pulmonary function or damage to the renal circulation affects 02 delivery to the kidney (Hoffbrand et. al, 2006).

1.1.2 leukopoiesis:

Are produced, which is a sub-process of hematopoiesis. Like other blood cells, white cells are originated from a pool of hematopoietic stem cells. Under the action of mainly G-CSF (Granulocyte Colony Stimulating Factor), a growth factor only acting on the leukocyte line, hematopoietic stem cells differentiate in progenitors (the so-called CFU, Colony Forming Units), which in turn will produce precursor cells after a consequent number of divisions. After a few divisions late, leukocytes are formed and leave the bone marrow to enter the bloodstream. Due to the number of divisions and the quantity of cells involved in leukopoiesis (or in hematopoiesis in general), issues may arise at different cellular levels and sometimes result in diseases affecting white cells. Among a wide variety of diseases affecting leukocytes, cyclical neutropenia is of great interest. It is characterized by a periodic decrease in the circulating neutrophil (white cells) numbers, from normal to low values, sometimes barely detectable (Haurie, 1998). Usually periods observed vary between 19 and 21 days, but longer periods up to 46 days have been reported in some patients. Oscillations of all leukocyte types (other than neutrophils) have been observed in patients with cyclical neutropenia, usually with the same periods. The understanding of cyclical neutropenia has been greatly aided by the discovery that a canine race, the grey collie, gets this

congenital disease (with rather shorter periods, in the order of 11 to 16 days) (Haurie, 1999).

1.1.3 Thrombopoiesis:

Platelets are produced predominantly by the bone marrow megakaryocytes as a result of budding of the cytoplasmic membrane. Megakaryocytes are derived from the haemopoetic stem cell, which is stimulated to differentiate to mature megakaryocytes under the influence of various cytokines, including thrombopoietin (Drew, 2003). The first step in assessing a patient's platelet function, especially if he or she presents with a bleeding problem, is to perform a platelet count. This can be done with a hemocytometer, but more commonly it is performed with an electronic particle counter. Although thrombocytopenia is defined as a count of less than 150, 000 platelets/μL, bleeding problems do not usually appear with counts above 50,000 platelets/μL, unless there is a functional defect as well. It is important to confirm thrombocytopenia by repeating the count using citrated blood and by examining a slide. Life span of ~10 days, a blood volume of 5 L, and one third of platelets pooled in the spleen, each day the average adult human must produce ~1 × 1011 platelets to maintain a normal platelet count under steady-state conditions, a level of production that can greatly increase under conditions of increased demand.Thrombopoiesis is dependent on the marrow microenvironment, composed of both cells and extracellular matrix proteins, and cell surface and soluble hematopoietic growth factors (Tavassoli, 1998).

Hepatic production of Tpo is largely constitutive, with mRNA and protein levels unresponsive to alterations in platelet count.10 In contrast, in patients with reactive thrombocytosis secondary to inflammation, IL-6 is responsible for enhanced hepatic production of Tpo and enhanced thrombopoiesis(Wolber,200:and Kaser,2001). While the importance of hepatic Tpo is clear, marrow stromal cell production of the hormone is less well established. However, multiple lines of evidence have established that the marrow stroma can produce Tpo, and that thrombocytopenia induces a striking increase in Tpo mRNA and protein. (Sungaran, 2000). Moreover, the molecular mechanisms that regulate this process have been traced to platelet granule proteins, which serve to inhibit stromal cell transcription of the Tpo gene.13-14 The sites in the Tpo gene responsible for its transcriptional regulation are beginning to be discerned (McIntosh, 2008).

1.2 Complete blood count (CBC)

A complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) or blood panel, is a test panel requested by a doctor or other medical professional that gives information about the cells in apatient's blood. A CBC is routinely performed during annual physical examinations in some jurisdictions. The CBC includes determinations of the hemoglobin, hematocrit, red blood cell count, red blood cell volume; and

hemoglobin content, platelet count, and white blood cell count. These measurements are provided by any of the common automated counters, including instruments manufactured by Abbott, Bayer, Beckman-Coulter, TOA, and Technicon A CBC also helps him or her diagnose conditions, such as anemia, infection, and many other disorders. (Dacie and Lewis, 2006).

1.2.1 Red Blood Cell (RBCs) count:

The normal erythrocyte has a diameter of about 8 µm and a biconcave disc form that provides the red cell with a maximum surface-for-gas exchange as well as optimal deformability. The bipolar lipid layer of the red cell membrane is stabilized on the inner side by the attachment of the structural proteins actin and spectrin. Defects of these proteins lead to hemolytic anemia. The outer layer is covered with mucopolysaccharides that form part of the structure of blood group antigens. The N-acetylneuraminic acid found in these glycoprotein's results in a negative charge of the cell surface. Because red cells have lost their nuclei, they are no longer capable of synthesizing proteins, including enzymes. Red cells remain viable and functional for an average of 120 days. The necessary energy for red cell metabolism is supplied by the Embden-Meyerhof pathway, which generates adenosine triphosphate by metabolizing glucose to lactate. This anerobic process also results in the formation of nicotinamide-adenine dinucleotide, which is essential for the reduction of methemoglobin to functionnally active hemoglobin (Reinhold, 2007).

Table 1-2. The Complete Blood Count (CBC) (William, 2002).

Component	Reported Units
RBC count	10^6 cells/_L
Hemoglobin	g/dL
Hematocrit Volumes	%
Mean corpuscular volume (MCV)	fL
Mean corpuscular hemoglobin (MCH)	Pg
Mean corpuscular hemoglobin concentration (MCHC)	g/dL
Red cell distribution width (RDW)	%
WBC count	10^3 ells/L
Neutrophils (segs + bands)	%10^3 cells /L
Lymphocyet	%10^3 cells /L
Monocytes	%10^3 cells /L
Eosinophils	%10^3 cells /L
Basophils	%10^3 cells /L
Platelet count	10^3 ells/L

The most important values of the CBC are listed in bold type

1.2.2 Haemoglobin (Hb):

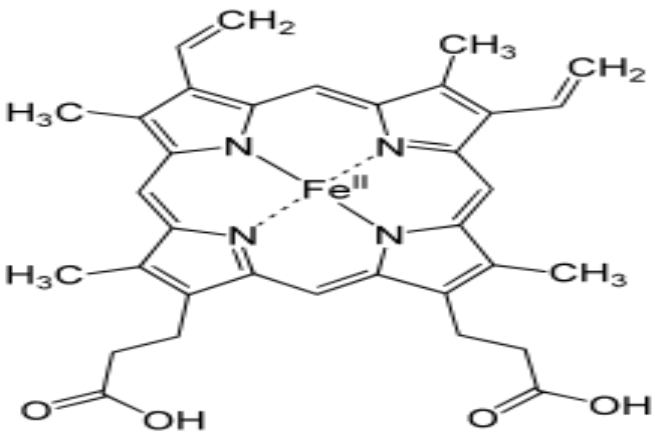
Haemoglobin is the iron-containing oxygen-transport metalloproteinase in the red blood cells of all vertebrates (Maton et. al, 1993). The main function of the red cells is to carry 02 to the tissues and to return carbon dioxide (C02) from the tissue to the lungs. Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin , in which CO2 is

bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen (Connie et. al, 1998). Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantianigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism (Bain, 1985).

1.2.2.1 Structure of haemoglobin:

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins (Van Kessel et.al, 2003). Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments (Figure 1-2). Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement (Hoff brand et. al, 2006).

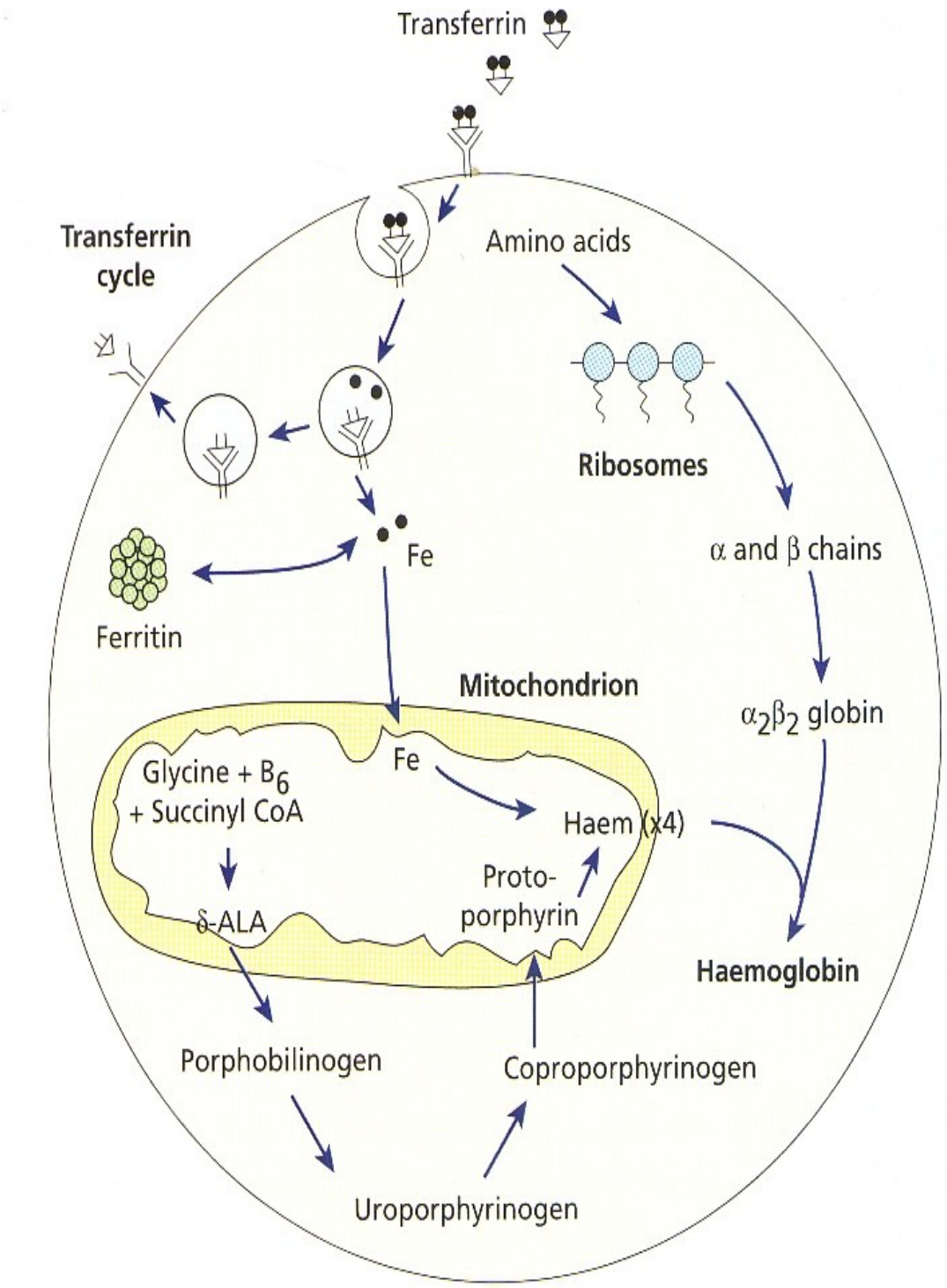
Figure 1-2. Structure of haemoglobin (William, 2002).



1.2.2.2 Synthesis of haemoglobin:

Hemoglobin (Hb) is synthesized in a complex series of steps. The hem part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosome's in the cytosol. Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this hemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name) (Hoffbrand et. al, 2006). Figure 1-3.

Figure 1-3. Synthesis of heamoglobin (Hoffbrand et. al, 2006)



1.2.2.3 Types of Hb in humans:

Hemoglobin variants are a part of the normal embryonic and fetal development, but may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics. Some well-known hemoglobin variants such as sickle-cell anemia are responsible for diseases, and are considered hemoglobinopathies. Other variants cause no detectable pathology, and are thus considered non-pathological variants (Huisman , 1996).

In the embryo:

1- Gower 1 (ζ2ε2)

2- Gower 2 (α2ε2) (PDB1A9W)

3- Hemoglobin Portland I (ζ2γ2)

4- Hemoglobin Portland II (ζ2β2).

In the fetus:

1- Hemoglobin F (α2γ2) (PDB1FDH).

In adults:

1- Hemoglobin A (α2β2) (PDB1BZ0) - The most common with a normal amount over 95%.

2- Hemoglobin A2 (α2δ2) - δ chain synthesis begins late in the third trimester and in adults, it has a normal range of 1.5-3.5%.

3- Hemoglobin F (α2γ2) - In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease and beta-thalassemia.

Elevated levels of hemoglobin are associated with increased numbers or sizes of red blood cells, called polycythemia. This

elevation may be caused by congenital heart disease, corpulmonale, pulmonary fibrosis, too much erythropoietin, or polycythemia Vera (Hoff brand et. al, 2006).

1.2.3 Hematocrit (HCT):

Hematocrit, the proportion of blood volume occupied by red blood cells, is typically about three times the hemoglobin concentration measured in g/dL. For example, if the hemoglobin is measured at 17 g/dL that compares with a hematocrit of 51% .The packed cell volume (PCV) can be used as a simple screening test for anemia, as a reference method for calibrating automated blood count systems, and as a rough guide to the accuracy of haemoglobin measurements. The haematocrit ×1000 is about three times the haemoglobin expressed in g/l. In conjunction with estimations of haemoglobin and red blood cell count (RBC), it can be used in the calculation of red cell indices. However, its use in under-resourced laboratories may be limited by the need for a specialized centrifuge and a reliable supply of capillary tubes (Dacie and Lewis, 2006).

1.2.4 Leukocytes (WBC):

The total WBC is determined in whole blood in which red cells have been lysed. The lytic agent is required to destroy the red cells and reduce the red cell stroma to a residue that causes no detectable response in the counting system without affecting

leucocytes in such a manner that the ability of the system to count them is altered (Dacie and Lewis, 2006).

Several types of leukocytes, or white blood cells (WBCs), are found in the blood. The normal WBC count is ~4,000 to 10,000/_L (4.0–10.0 _103/_L). Leukocytes are usually divided into granulocytes, which have specific granules, and granulocytes, which lack specific granules. Granulocytes are divided into neutrophils (with faintly staining granules), eosinophils (with large reddish or eosinophilic granules), and basophiles (with large dark blue or basophilic granules). Granulocytes are divided into lymphocytes and monocytes (William, 2002).

1.2.4.1 Neutrophils:

Neutrophils are the most common type of WBCs in adults. Two types are described: segmented neutrophils and band neutrophils:

1- Segmented neutrophils (“segs,” also called polymorph nuclear neutrophil Leukocytes [PMNs or “polys”]) have a nucleus divided into multiple distinct lobes connected by thin strands of chromatin. The cytoplasm has fine granules that stain lightly with the usual blood stains. Polys normally comprise ~50 to 70% of total WBCs.

2- Band neutrophils (“bands,” sometimes called “stabs”) have a horseshoe-shaped nucleus, without the distinct lobes of polys. They are an earlier stage than segmented neutrophils but are

fully functional. Bands normally represent ~2 to 6% of all WBCs; the number of bands increases with acute stress or infection.

The primary function of neutrophils is phagocytosis, predominantly of bacteria; neutrophils are the primary defense against bacterial infection. Bacteria are killed by antimicrobial agents contained or generated within neutrophil granules. Neutrophils circulate in the blood for ~10 hours and may live 1 to 4days in the extra vascular space. The trip is one way; once neutrophils leave the blood to enter tissues, they cannot return. A significant number of neutrophils are rolling along the endothelial surface of blood vessels (the marinating pool).This population can be rapidly mobilized with acute stressor infection (Reinhold and Erhard, 2007).

1.2.4.2 Eosinophils:

These cells are similar to neutrophils, except that the cytoplasmic granules are coarser and deeper lyred staining and there are rarely more than three nuclear lobes. (Eosinophil myelocytes can be recognized but earlier stages are indistinguishable from neutrophil precursors. The blood transmit for eosinophils is longer than for neutrophils.They enter inflammatory exudates and have a special role in allergic responses, defense against parasites and removal of fibrin formed during inflammation (Hoffbrand et. al, 2006).

1.2.4.3 Basophils:

Basophils are seen less frequently than eosinophils; under normal conditions, fewer than 100 cells/μL are found in the peripheral

blood. Basophils have receptors for immunoglobulin (Ig) E and, in the cytoplasm; characteristic dark granules overlie the nucleus. Degranulation of basophiles results from the binding of IgE and allergic or anaphylactic reactions are associated with the release of histamine and heparin (Reinhold and Erhard, 2007).

1.2.4.4 Lymphocytes (“Lymphs”):

Lymphocytes are the second most common type of leukocytes in adults (~20–40% of WBC). The lymphocyte number is higher in children and also increases with viral infections.

Functionally, there are two main types of lymphocytes: B cells and T cells.

B Cells:

1- B cells are the primary effectors of the humoral (antibody-mediated) Immune system.

2-They develop in the bone marrow and are found in lymph nodes, the spleen and other organs, as well as the blood.

3-After antigen stimulation, B cells may develop into plasma cells, which are the primary antibody-producing cells.

T Cells:

1- T cells are the main effectors of cell-mediated immunity.

2-T cells are the command and control cells of the entire immune system: they stimulate or inhibit the function of other cells of the immune system, including B cells, monocytes and macrophages, and other T cells.

3-T cell precursors originate in the bone marrow but develop and mature in the thymus (T = thymic dependent).

4-Normally, the majority of circulating lymphocytes are T cells.

5-T cells are divided into two main subtypes:

6-T helper cells, which are the major regulatory cells of the immune system, usually express a surface antigen designated CD4.

7-T suppressor/cytotoxic cells are involved in the destruction of virally infected cells and rejection of transplanted (William, 2002).

1.2.4.5 Monocytes (“Monos”):

Monocytes normally comprise ~3 to 8% of leukocytes. After 8 to 14 hours in the blood, they enter tissue to become tissue macrophages (also called histiocytes). Monocytes are large cells, with abundant light gray to light blue finely granular cytoplasm. The nucleus appears as a horseshoe-shaped structure with chromatin that is less dense than that seen in mature neutrophils. The cytoplasm is a grayish-blue color and contains sparse numbers of pink to purplish granules. Histochemically, monocytes are best identified by their strong staining with nonspecific esterase

Monocytes have two functions:

1- Phagocytosis of microorganisms (particularly fungi and mycobacteria) and debris.

2- Antigen processing and presentation. In this role, they are critical in initiation of immune reactions (Hillman et. al, 2005).

1.2.5 Platelet count:

The normal circulating platelet count is maintained within relatively narrow limits (150,000 - 450,000 platelets/ĺμL in

Northern Europeans and 90,000 - 300,000 platelets/ĨμL in people of Mediterranean descent). The platelet volume is inversely related to the platelet count, so the mass of circulating platelets is the same for these two populations. Approximately one-third of platelets are sequestered in the spleen at any one time. Since a platelet has a lifespan of approximately 9 to 10 days, some 15,000 - 45,000 platelets/ĨμL must be produced each day to maintain a steady state (Dacie and Lewis, 2006).

A normal citrate count or evidence of platelet clumps under the microscope would suggest pseudo thrombocytopenia, which is caused by an effect of EDTA on the platelet and appears to have no clinical significance (Martin et. al, 2005). In health, there are approximately 150 – 400 × 109 platelets per litre of blood. The counts are somewhat higher in women than in men (Bain, 1985), and there is a cycling, with slightly lower count at about the time of menstruation. Lower platelet counts have been observed in apparently healthy West Indians and Africans than in Caucasians (Bain and Seed, 1986).

1.2.6 Red cell indices:

Red cell indices traditionally have been the derived parameters of MCV, MCH, and MCHC; more recently, red cell distribution width (RDW) has also been included and, for some instruments, haemoglobin distribution width (HDW). These indices are the basis for classifying anaemias, and in various combinations they have been used to aid in the distinction between iron deficiency and thalassaemias (Lafferty et. al, 1996).

1.2.6.1 Men cell volume (MCV):

MCV is measured directly, but in semi automated counters MCV is calculated by dividing the PCV by RBC (femtoliters).The MCV has been used to guide the diagnostic workup in patients with anemia, for example testing patients with microcytic anemia for iron deficiency or thalassemia, 16 and those with macrocytic anemia for folate or vitamin B12 deficiency (Griner and Oranburg, 1978).

1.2.6.2 Men cell heamoglobin (MCH):

The MCH, the amount of hemoglobin per red cell, is calculated by the formula MCH (pg/cell) = hemoglobin (g/dl) / red cell count (x 106 ells/1) x 10.The MCH increases or decrease as the does the MCV and generally provide little additional diagnostic information (Williams, 2002).

1.2.6.3 Men cell heamoglobin concentration (MCHC):

The MCHC, the concentration of hemoglobin per unit with red cell volume, is calculated by the formula MCHC (g/dl of red cells) = hemoglobin (g/dl) / hematocrit (ml/100 dl) x 100. An MCHC greater than 35 g/dl red cells is associated with hereditary spherocytosis,23 and a low MCHC is typical of iron deficiency,24 but its diagnostic usefulness is limited (Mahu et. al,1990).

1.2.7 Red cell distribution width (RDW):

The red cell distribution width (RDW) is specifically designed to reflect the variability of red cell size. It is based on the width of

the red blood cell volume distribution curve, with larger values indicating greater variability. An elevated RDW may be an early sign of iron-deficiency anemia (Dacie and Lewis, 2006), and although proposed as an aid in distinguishing iron deficiency from other causes of microcytic anemia, such as thalassemia, the RDW is not sufficiently specific to obviate the need for more specific tests. The RDW can be used in the laboratory as a flag to select those samples submitted for automated blood count that should have manual review of the blood film for red cell morphology (Flynn et .al, 1986).

1.2.8 Morphologic Examination of the Blood:

Microscopic examination of the blood spread on a glass slide or cover slip yields useful information regarding all the formed elements of the blood. The process of preparing a thin blood film causes mechanical trauma to the cells. Also, the cells flatten on the glass during drying, and the fixation and staining involve exposure to methanol and water. Some artifacts are inevitably introduced, but these can be minimized by good technique (Dacie and Lewis, 2006).

Peripheral blood smear: The ideal PBS requires a fresh drop of capillary blood without anticoagulation. However, a fresh venous sample collected in EDTA is satisfactory and as it is convenient is widely used. A drop of blood is placed on one end of a clean glass slide and is spread either mechanically or manually resulting in a tongue or bullet shaped smear covering 1/2-2/3 of the slide. The slide is rapidly air dried, fixed in methanol and stained typically

with Wright-Giemsa. The examination starts with a macroscopic view to evaluate the quality of the smear based on overall appearance. The microscopic analysis begins on lower power (10x), primarily to assess cellular distribution, staining quality, and to select an area where the RBCs are barely touching each other. This area is used to conduct a complete assessment of the cellular elements on higher magnification.

On hi-dry (40x), the slide is principally scanned to obtain a WBC estimate. All of the detailed analysis of the cellular elements is performed using oil immersion. This final microscopic examination is performed at 50x or 100x oil immersion and includes:

• A WBC differential
• The identification of abnormal or peculiar leukocytes
• Assessment of RBC morphology
• The number and morphology of the platelets
• The identification of intra- and extra-cellular elements

Blood films should be examined in a systematic way. First the Film should be examined without using the microscope, to make sure it is well spread (not too thick, too long or too short) and that its staining characteristics are normal. A film that is a deeper blue than other films stained in the same batch is usually indicative of an increase in the concentration of plasma proteins. (Barbara, 2004).

Table 1-3. Approximate normal blood values (Dacie and Lewis, 2006).

Value	Male	Female
Hemoglobin(g/dl)	13-18 g/dl	12-16 g/dl

PCV %	40-54%	37-47%
RBC 10^{12}/l	4.7-6.1$10^{12}$/l	4.2-5.4$10^{12}$/l
WBC 10^{9}/l	4.0-10.0 10^{9}/l	
Platelet count 10^{9}/l	145-400 10^{9}/l	
MCV(fl)	80-96 fl	
MCH (pg)	27-32 pg	
MCHC (g/dl)	32-36 g/dl	
RDW %	9.5–15.5%	
Neutrophils	36-75	
Lymphocytes	20-50%	
Monocytes	3-8%	
Eosinophils	0-5%	
Basophils	0-2%	

1.3 Khat

1.3.1 Definition:

Khat is a name generally used for Catha edulis, adisctyledonous evergreen shrub of the family celastraceae (kennedy et. al, 1987a). Khat (Catha edulisforsk) is an evergreen shrub of the

celastraceae family, normally reaching 6m in height, but in an equatorial climate it might grow to 25cm (Unodc,1956) Also spelled qat ,Kat,cat or ghat:the amharas call it tchat and the Gallas,Jimma:in Kenya khat is known as (miraa: Khat is probably the most correct transliteration of the Arabic word (Peters,1953).

1.3.2 Description:

Khat is as low-growing tree that grows to between 1.5meters and 20meters tall, depending on region and rain fall, with evergreen leaves 5-10cm long and 1-4cm broad. In the Yemen Republic, about 44 different types of khat exist originating from different geographic areas of the country (Geisslusler and Brenneisen, 1987).

1.3.3 Prevalence of khat chewing:

Khat use is highly prevalent in East African and Middle Eastern countries, in particular the Yemen (Manghi, 2009).15 years ago, Kalix mentioned 6 million daily khat users (Kalix, 1984). Khat chewing among male Aden University medical students increased from 35% to 90% over the 5 years of training (Laswar and Darwish, 2009). In college and high school students of Jazan region of Saudi Arabia aged between 15 and 25 years, 37.7% of males and only 3.8% of female's chewed khat (Ageely, 2009). 15.9% of a sample of 4001 men in Addis Ababa, Ethiopia regularly chewed khat (Tesfaye et. al, 2008). There is evidence that khat use in Ethiopia is more prevalent in ethnic com- munities with a tradition of khat use but it is now becoming an every-day drug for

the general population (Belew et. al, 2000). The prevalence of khat chewing in Western countries appears to be restricted to the immigrant communities from these countries where there is still a high prevalence among the immi- grant groups (Manghi, 2009). In the UK, 75 male Yemeni adults reported chewing up to 3 bundles of khat per week of which 39% were assessed as dependent (Kassim et. al, 2006). In Somali communities of the UK, approximately 1 third chews khat on a reg- ular basis (Patel et. al, 2005).

1.3.4 Habit description:

Chewing the leaves of khat (Catha edulisForsk.) is a social habit in Yemen and East African countries. People chew fresh khat leaves daily on a regular basis mainly in the afternoon, although some people start to chew khat in the morning. Khat is usually chewed at special social gatherings, but is also used frequently during work by labourers, craftsmen, farmers and students to keep alert and reduce physical fatigue (Manghi, 2009).The habit has a deep-rooted social and cultural tradition, particularly in Yemen (Kalix and Braenden,1984). The social khat session, in Yemen called majlis al- khat, is held in the afternoon in a special warm reception room for khat -chewing. Guests sit comfortably and chew the fresh leaves one by one. The juice is swallowed while the residue is retained as a quid against the cheek on one side of the mouth; a quantity of 100 – 200 g is usually consumed (Kalix, 1996).Initially, the session is lively and as the alerting effects of khat start to work, the session becomes more serious and the

khat (Catha edulis)—an updated review 301chewers' talk focuses on one subject at a time. The topic may be a current world event, a historical or religious issue, apolitical situation or a local dispute. After 2 – 3 hours the session becomes quiet as most of the chewers prefer to be left alone, falling into intense concentration and mental focus. After about 4 hours, people start to depart the session. A detailed description of a typical khat session is provided elsewhere khat chewing is predominantly a male habit, but women also practice it. The khat session described above is typical for male khat chewers, for whom the chewing is the main event. The female khat sessions, however, are held less frequently and the social gathering itself, in some areas of Yemen called tafruta, is more important than chewing; much smaller quantities of khat are chewed and for shorter periods (Kennedy,1987e). The habit is limited ainlytoold and married women, as it is not socially accepted for young unmarried women to chew khat (Al-Motarreb et. al, 2002a).

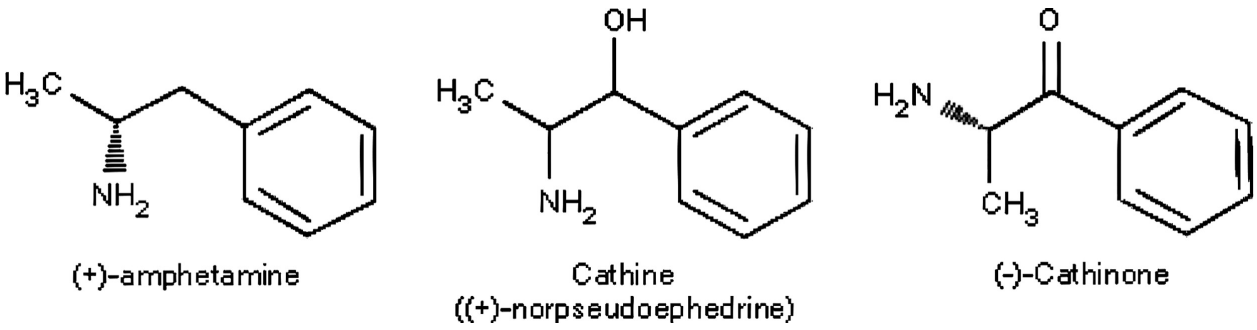
1.3.5 Constituents of khat :

There are three main alkaloids present in khat leaves S-(-)-cathinone (S- - alpha-minopropropriphenon) nor pseudo ephedrine (cathine) and nor ephedrine. There are also small amounts of ethereal oil, sterol and triterpenes, to gather with 5% protein which has in significant nutritional value. Ascorbic acid is also present in the leav (Kennediy et. al, 1980). Recently analysis using liquid chromatography mass spectrometry revealed the presence of 62 cathedulin alkaloids in the crude methanolic

extracts of fresh khat (Kite et. al, 2003). Later studies showed that cathinone is present at a high concentration in the neither young leaves, while being converted rapidly in the adult leaves into cathine and, to a lesser extent, into nor ephedrine: another phenyl alkylamine described in khat. Both cathine and cathinone are related structurally to amphetamine (Zelger et. al, 1980). Figure 2- 1

Figure 2- 1 Chemical structure of amphetamine, cathine and cathinone.

.



1.3.6 Effect of khat:

The chewing of khat leaves has involved at least 80% of adult males and extended to women, too (Manghi, 2009). The WHO 2003 to 2006 reported that khat consumption has become a common problem that affects the health aspects of life (Kassim, 2010). In fact, many adverse effects have been associated with khat consumption. (Al-Motarreb et. al, 2010).Accordingly, prolonged exposure to khat could result in psychoneurological disturbances such as neurosis (Hoffman and Al'Absi, 2010). In

addition, increased diastolic blood pressure (Getahun, 2010), and vasoconstriction of coronary vasculature were also reported (Zubaid et. al, 2010). More commonly, gastritis (Nencini and Ahmed, 1998), hemorrhoids, and duodenal ulcer had a higher prevalence among khat chewers (Manghi, 2009). Furthermore, Luqman and Danowski reported that liver cirrhosis that was observed among Yemeni khat chewers might be due to khat consumption, but at that time it was not further investigated (Luqman and Danowski, 1976), and hepatotoxicity of khat chewing is still debated in humans (Coton et. al, 2011). In animals, the administration of crude extract of khat to New Zealander white rabbits for three months suggested toxic hepato cellular jaundice as well as histopathological abnormalities in livers of such animals (Al-Mamary et. al, 2002). Likewise, a companion study on the same species of animals with the same dose levels of crude extract for six months supported the former three-month findings; however, after six months histopathological evidence from liver sections suggested per portal fibrosis as an initial indicator of liver cirrhosis without apparent damage to kidneys (Al-Habori et. al, 2002). As a limitation, those studies did not include females for the hepatotoxicity studies and evaluation of khat ne phrotoxic effects in either males or females was only minimally investigated. In addition, the doses of crude extract that were given to animals were selected to be in the average of 150–200 g of fresh khat leaves according to Kalix (Kalix, 1985), which was less than that suggested by Al-Habori et. al. (Al-Habori

et. el, 2002). However, there are no comparative studies about the effects of the different dose levels of khat on human in terms of body weight. Recently, several reports were published on severe liver diseases in khat users i n Yemen (Peevers et. al, 2010).

This finding suggested that a constituent of khat attenuates the ant platelet aggregating proper- ties of aspirin, there by neutralizing the beneficial actions of aspirin. Adrenaline induces aggregation of human platelets which is mediated via alpha 2-drenoceptors (Broadley, 1996). The (El Hadrani et. al, 2000), that reported khat consumption has no effect in all CBC parameters.

In Yemen, khat farmers use many pesticides randomly for spraying khat trees. The most common pesticides used in Yemen are orgnophosphorus such as Dimethoate,parathion,Diazinon,Chlorpyrifos Imidacloprid 10% WP,Hexaconazole and Bifenthrin. The (Varol and Gultkin, 2012), has found that effect of pesticides exposure on platelet indices in farm workers on platelets count, MPV and PDW.

In addition (Krug and Berudt, 1985) has been found that effect of pesticides on platelet aggregation and archiadonic acid metabolism.

1.4 Rationale

The chewing of khat leaves has involved at least 80% or more of adult males and extended to women, too. The WHO 2003 to 2006 reported that khat consumption has become a common problem that affects the health aspects of life (Kassim, 2010). The studies concerning khat effect on CBC were rare accordingly, this work has been conducted. However, in Yemen, in the absence of law and monitoring, this might be a reason for the appearance of diseases due to more pesticides not documents in agriculture ministry. Therefore, this is the first study from Yemen, aimed to find out the effect of khat chewing on CBC parameters.

1.5 Objectives

1.5.1General objective:

- To measurements of Effect khat consumption on complete blood counts in Sana’a city.

1.5.2 Specific objectives:

1 - To evaluate the full blood count in people khat chewing in Yemen.

2 - To compare complete blood counts of chewing khat individuals with non-chewing khat individual (control).

3 - To correlate between CBC on one hand and duration of khat chewing age of participant and doses of khat.

Chapter two

Materials and Methods

2.1 Study design:

This study design is case control studies were conducted to obtain clear information about the disturbances of haematological parameter due to khat chewing in Yemen.

2.2 Study area:

This study was conducted in unit blood bank of typical police hospital - Sana'a city, Yemen .During period from April 2013 to April 2014.

2.3 Study population:

The study performed on 329 healthy volunteers (male and femal) aged between 18- 65 years.

They will be divided into two groups:

- **Group A:**

1- Healthy people chewing khat (n=177).

2- Healthy people chewing khat with smoking (n=92).

- **Group B:**

Non-chewing khat and smoking (n=60). (Control)

2.3.1 Criteria of study subjects:

2.3.1.1 Inclusion criteria:

1- Chewing khat for period more than two years.

2- Chewing khat at least 2-4hours/day or above.

2.3.1.2 Exclusion criteria:

1- Children and patient with illness aneamia, lukeamia and other bleeding disease.

3- Exclude any patient with treatment.

2.4 Sample size:

In this study sampling was anon-probability sample 329 sample was chosen randomly 269 were people chewing khat and 60 as control.

2.5 Method of sample collection:

2.5.1 Sample collection

Blood sample (2.5ml) were taken from antecubital vein into EDTA, container according to local ethics of Sudan university, written informed consent from all patients were obtained and the following investigation were carried out to each sample Hb,HCT,RBCs,WBCs, Plts count, blood stained in blood film.

People was lied on khat chewing the arm were positioned in comfortable situation and the tourniquet were applied gently to avoid venous stasis ,then arm was cleaned by 70%alcohol and the

needle were inserted and 5ml of blood were collected (Lewis2006).

2.5.2 Principle of automated haematological analyzer system (CBC):

blood cell and particles can be counted based on either electrical impendence or light scatter technique ,the automated blood counter analyzer has a multiple channels in one channel ,the diluents are added and the red cell is counted and it size will be determine in the other channel ,the lytic agent is added together with diluents to covert the red cell into stroma, leaving white cell intact for counting and also producing a solution in which the hemoglobin can be measured, further channels are required for differential WBCs counts. (Lewis et al, 2006).

Procedure

The reagent required for operating are checked then the power switch were turn on auto rinse and background check were of automatically performed then three level of control (low count ,normal count and high)were applied after selection of whole blood mode of analysis sample number were introduce by pressing sample number keys then enter key was pressed after that the sample mixed carefully the tube was bring in close contact with sample probe and the start key were pressed the required volume of blood were aspirated when the LCD screen display analyzing the tube were removed after that the automatic

analysis were done and the result was displayed in the screen then the result was printed (Sysmex America INC 2003)

2.6 Ethical consideration:

Each individual was told about important of the study during the interview and all of them agreed to participate in this study. The proposal of this study was approved by the Faculty Research Board, Faculty of Medical Laboratory, and Sudan University for Science and Technology.

2.7 Data analysis:

The data were analyzed by the SPSS 16.0 (Social Package of Statistical Science) computer program by LEAD Technologies; Inc. USA (1991-2000) and the results were expressed as Mean ± SD and compared by independent sample T-test & Anova test. The significant interrelationships between parameters were analyzed by Pearson correlation. The significant differences were indicated if P-value was < 0.05.

Chapter Three

Results

Thirty hundred and twenty nine (329) volunteer donors were enrolled for this study, of whom 269 were khat chewers (cases) and 60 were controls, their ages ranging from 18 to 60 years, the mean age was 32 years old. The age distribution was similar among the cases and the controls. The bulk of the cases and controls belonged to the young age range 18–23 years, constituted 59.5% of cases and 51.7% of controls, (Table 3-1). Both genders participated in this study, males constituted (87.8%) and females constituted (11.9%) in the study population.

In distribution of CBC components study population with khat chewing and control groups, the following results were indicated in Table 3-2. The mean of hemoglobin level (15.86±1.59) for khat chewing and (15.50±1.05) for control, there was no statistical difference (P=0.099), as seen in Table 3-2.The mean of PCV (47.70±4.59) for khat chewing and (46.73±3.53)for controls, also there was no statistical difference (P=0.122) ,as seen in Table 3-2..The mean of red blood cell (5.47±o.74) for khat chewing and (5.47±0.39) for controls (P=0.993) ,as seen in Table 3-2. The mean of white blood cell (6.48±1.97) for khat chewing and (6.72±1.77) for controls (P=0.388), as seen in Table 3-2.The mean of platelets was 285.13±80.93 for khat chewing and 312.26±67.31 for controls, the effect was found to be statistically different (P=0.013), as seen in Table 3-2. The mean of nutrophil, lymphocyte and monocyt (49.82±12.34), (38.89±11.41) and (6.59±2.34) for khat chewing and (50.41±11.34), (39.85±10.77)

and (6.33±2.10) for controls, with P values of, (P=0.734), (P=0.555) and (P=0.428) respectively, as seen in Table 3-2.

The mean of eosinophil (4.44±3.54) for khat chewing and (3.26±2.63) for controls, the effect was found to be statistically different (P=0.016) as seen in Table 3-2.

The mean of MCV, MCH, MCHC (84.82±4.23), (28.61±1.55) and (32.89±0.89) for khat chewing and (84.41±3.84), (28.61±1.16), (32.83±0.61) for controls, with P values of (P=0.493), (P=0.998) and (P=0.656), respectively, as seen in Table 3- 2. The mean RDW (14.38±0.84) for khat chewing and (14.22±.67) for controls, (P=0.167), as seen in Table 3-2.

In distribution of CBC Components in khat chewing in association with smoking, the following results were explained in Table 3- 3. The mean of hemoglobin level (16.30±1.51) for khat chewing and (15.63±1.59) for control, (P=0.001), as seen in Table 3-3.The mean of PCV (49.00±4.25) for khat chewing and (47.02±4.62) for controls, the effect was found to be statistically significant (P=0.001) ,as seen in Table 3- 3.The mean of red blood cell (5.60±0.62) for khat chewing and (5.40±.78) for controls the effect was found to be statistically significant (P=0.031) ,as seen in Table 3- 3. The mean of white blood cell (6.64±1.83) for khat chewing and (6.40±2.04) for controls, (P=0.358), as seen in Table 3- 3.The mean of platelets (276.97±72.25) for khat chewing and (289.32±80.93) for controls, (P=0.220), as seen in Table 3- 4.The mean of nutrophil, lymphocyte, monocyt and eosinophil (49.60±12.19), (39.02±11.52), (6.39±2.20) and (4.75±3.93) for

khat chewing and (49.93±12.44), (39.08±11.32), (6.70±2.41) and (4.28±3.31) for controls, respectively,(P=0.836), (P=0.897), (P=0.307) and (P=0.311) respectively, as seen in Table 3- 3.

The mean of MCV, MCH and MCHC (85.23±4.37), (28.63±1.42) and (33.00±1.26) for khat chewing and (84.61±4.15), (28.61±1.61), (32.83±0.89) for controls, respectively, (P=0.249), (P=0.919) and (P=0.194), respectively, as seen in Table3- 3. The mean RDW (14.49±0.76) for khat chewing and (14.32±0.88) for controls, (P=0.105), as seen in Table 3- 3.

In distribution of CBC components study population with khat chewing (smoking) and control groups, the following results were indicated in Table 3-4. The mean of hemoglobin level (16.30±1.59) for khat chewing and (15.50±1.05) for control, the effect was found to be statistically significant (P=0.001), as seen in Table 3-4.The mean of PCV (49.00±4.25) for khat chewing and (46.73±3.53)for controls, the effect was found to be statistically significant (P=0.001) ,as seen in Table 3-4..The mean of red blood cell (5.61±o.61) for khat chewing and (5.47±0.39) for controls (P=0.116) ,as seen in Table 3-4. The mean of white blood cell (6.64±1.83) for khat chewing and (6.72±1.77) for controls, (P=0.776), as seen in Table 3-4.The mean of platelets (276.91±72.38) for khat chewing and (307.40±66.28) for controls, the effect was found to be statistically significant (P=0.010), as seen in Table 3-4. The mean of nutrophil, lymphocyte and monocyt (49.60±12.19), (39.02±11.26) and (6.39±2.20) for khat chewing and (50.41±11.34), (39.85±10.77),

(6.33±2.10) and for controls, respectively, (P=0.682), (P=0.653) and (P=0.872) respectively, as seen in Table 3-4. The mean of eosinophil (4.75±3.93) for khat chewing and (3.26±2.63) for controls the effect was found to be statistically different (P=0.011), as seen in Table 3-4.

The mean of MCV, MCH, MCHC (85.23±4.37), (28.63±1.42) and (32.67±3.37) for khat chewing and (84.41±3.84), (28.61±1.16), (32.83±0.61) for controls, respectively, the effect was found to be no statistical difference (P=0.237), (P=0.950) and (P=0.718), respectively, as seen in Table 3- 4. The mean RDW (14.49±0.79) for khat chewing and (14.22±.67) for controls, the effect was found to be statistically different (P=0.024), as seen in Table 3-4.

In distribution of CBC components study population with khat chewing (non smoking) and control groups, the following results were indicated in Table 3-5. The mean of hemoglobin level (15.63±1.59) for khat chewing and (15.50±1.05) for control, (P=0.561), as seen in Table 3-5.The mean of PCV (47.02±4.62) for khat chewing and (46.73±3.53) for controls, (P=0.946) ,as seen in Table 3-5.The mean of red blood cell (5.40±o.78) for khat chewing and (5.47±0.39) for controls, (P=0.501) ,as seen in Table 3-5. The mean of white blood cell (6.40±2.04) for khat chewing and (6.72±1.77) for controls, (P=0.281), as seen in Table 3-5.The mean of platelets (289.32±80.93) for khat chewing and (312.26±67.31) for controls, the effect was found to be statistically different (P=0.049), as seen in Table 3-5. The mean of nutrophil, lymphocyte, monocyt and eosinophil (49.93±12.44),

(38.83±11.52), (6.70±2.41) and (4.28±3.31) for khat chewing and (50.41±11.34), (39.85±10.77), (6.33±2.10) and (4.28±3.31) for controls, respectively, (P=0.793), (P=0.548), (P=0.296) and (P=0.32) respectively, as seen in Table 3-5.

The mean of MCV, MCH, MCHC (84.61±4.15), (32.83±0.89) and (32.83±0.89) for khat chewing and (84.41±3.84), (28.61±1.16), (32.83±0.61) for controls, respectively, (P=0.571), (P=0.977) and (P=0.980), respectively, as seen in Table 3- 5. The mean RDW (14.32±0.88) for khat chewing and (14.22±.67) for controls, (P=0.416), as seen in Table 3-5.

In distribution of duration of does to chews khat, from 2 to 12 years, 13 to 23 years and 24 to 36 years groups, the following results were observed; in 171(63.6%), 77(28.6%) and 21(7.8%) of the cases, respectively, the risk was not found to be statistically significant (P>0.05), excepted platelets was found to be statistically significant (P=0.037) as seen in Table 3 -6, 3-7.

Effects of khat chewing on CBC of both sexes was found significant statistically in Hb, PCV, and RBCs count (P=0.000) , the mean for male (15.85±1.47),(47.65±4.30),and(5.44±0.74), and mean for famel is (13.81±1.39),(41.80±3.94) and (4.61± . 70),respectively, as see in Table 3-8. And the effects of khat chewing on CBC of both sexes was not differed statistically in WBC (P=0.411), PLTs (P=0.667). MCV (P=0.617), MCH (P=0.492), and MCHC (P=0.139), and differential count (P>0.05), has been noticed Table 3-8.

In distribution of CBC Components in khat chewing with quantity, the following results were explained in Table3-9. The mean of hemoglobin level (15.66±1.55) for khat chewing and (15.95±1.61) for control, (P=0.169), as seen in Table 3-9.The mean of PCV (47.39±4.50) for khat chewing and (47.84±4.63) for controls, (P=0.448) ,as seen in Table 3-9.The mean of red blood cell (5.52±0.73) for khat chewing and (5.44±.74) for controls the effect was found to be no statistically significant (P=0.451) ,as seen in Table 3-9. The mean of white blood cell (6.30±1.95) for khat chewing and (6.57±1.98) for controls the effect was not found to be statistically significant (P=0.291), as seen in Table 3-9.The mean of platelets (293.13±69.59) for khat chewing and (281.32±81.79) for controls, the effect was not found to be statistically significant (P=0.248), as seen in Table 3-9. The mean of nutrophil, lymphocyte, monocyt and eosinophil (49.44±13.26), (39.44±11.88), (6.72±2.25) and (4.02±3.50) for khat chewing and (50.00±11.91), (38.63±11.21), (6.53±2.39) and (4.64±3.55) for controls, respectively, the effect was not found to be statistically significant (P=0.728), (P=0.592), (P=0.547) and (P=0.180) respectively, as seen in Table 3-9.

The mean of MCV,MCH and MCHC (84.8±3.97),(28.51±1.46) and (33.00±1.26) for khat chewing and (84.61±4.15),(28.61±1.61), (32.79±0..76) for controls, respectively, the effect was not found to be statistically significant (P=0.901),(P=0.446) and (P=0.245), respectively, as seen in Table 3- 9.The mean RDW(14.36±0.94)

for khat chewing and (14.39±0.80) for controls, the effect was not found to be statistically different (P=0.180),as seen in Table 3-9.
The correlation between CBC components and duration was found statistically no significant (p value >0.05) as seen table 3- 10.
The correlation between CBC components and does was not found to be statistically different (p value >0.05) as seen table 3- 10.
The correlation between CBC components and sex was not found to be statistically significant (p value >0.05) in Hb, PCV, RBC, and RDW as seen table 3- 8. And the correlation between CBC components and sex was not found to be statistically different (p value >0.05) in WBC, PLTS, differential whit blood, and red cell incidence (MCV, MCH and MCHC) as seen table 3- 10.

Table 3- 1 Distribution of case and control groups by age.

Age groups	Cases		Controls		Total	
	NO	%	NO	%	NO	%
18-23 years	160	59.5%	31	51.7 %	191	58.1%
33-46	83	30.9 %	25	41.7 %	108	32.8 %
47-60	26	9.7%	4	6.7 %	30	9.1 %
Total	269	100%	60	100%	329	100%

Table 3-2 CBC Components in study population with khat chewing and control.

CBC components	Cases	NO.	Mean±std	p-value (t-test)
Hp (gm/dl)	Khat chewing	269	15.86 ±1 . 59	0.099
	Control	60	15.50 ±1.05	
Pcv (%)	Khat chewing	269	47.70 ±4.59	0.122
	Control	60	46.73 ±3.53	
RBCs (/mm^3)	Khat chewing	269	5.47±o.74	0.992
	Control	60	5.47±0.39	
WBCs(mm^3)	Khat chewing	269	6.48±1.97	0.388
	Control	60	6.72±1.77	
PLTs(mm^3)	Khat chewing	269	285 ±78.15	0.013
	Control	60	312.26±67.3 1	
Nutro. %	Khat chewing	269	49.82±12.34	0.734
	Control	60	50.41±11.34	
Lymph. %	Khat chewing	269	38.89±11.41	0.555
	Control	60	39.85±10.7 7	
Mono. %	Khat chewing	269	6.59±2.34	0.428

	Control	60	6.33±2.10	
Eosn. %	Khat chewing	269	4.44±3.54	0.016
	Control	60	3.26±2.63	
MCV(femolite)	Khat chewing	269	84.82±4.23	0.493
	Control	60	84.41±3.84	
MCH (pg/cell)	Khat chewing	269	28.61±1.55	0.998
	Control	60	28.61±1.16	
MCHc(gm/dl)	Khat chewing	269	32.89±0..89	0.656
	Control	60	32.83±0.61	
RDW %	Khat chewing	269.	14.38±0.84	0.167
	Control	60	14.22±.67	

Table 3- 3 CBC components in Khat chewing in association with smoking.

CBC components	Smoking	NO.	Mean±std	p-value (t-test)
Hp (gm/dl)	Yes	92	16.30±1.51	0.001
	No	177	15.63±1.59	
Pcv (%)	Yes	92	49.00±4.25	0.001
	No	177	47.02±4.62	
RBCs (/mm^3)	Yes	92	5.60±0.62	0.031
	No	177	5.40±.78	
WBCs (mm^3)	Yes	92	6.64±1.83	0.358
	No	177	6.40±2.04	
PLTs (mm^3)	Yes	92	276.97±72.25	0.220
	No	177	289.32±80.93	
Nutro. %	Yes	92	49.60±12.19	0.836
	No	177	49.93±12.44	
Lymph. %	Yes	92	39.02±11.26	0.897
	No	177	38.83±11.52	
Mono. %	Yes	92	6.39±2.20	0.307
	No	177	6.70±2.41	
Eosn. %	Yes	92	4.75±3.93	0.311

	No	177	4.28±3.31	
MCV (femolite)	Yes	92	85.23±4.37	0.249
	No	177	84.61±4.15	
MCH (pg/cell)	Yes	92	28.63±1.42	0.919
	No	177	28.61±1.61	
MCHc (gm/dl)	Yes	92	33.00±1.26	0.194
	No	177	32.83±0.89	
RDW %	Yes	92	14.49±0.76	0.105
	No	177	14.32±0.88	

Table 3-4 CBC Components in study population with Khat chewing (smoking) and control.

CBC components	Cases	NO.	Mean±std	p-value (t-test)
Hp (gm/dl)	Khat chewing	92	16.30±1 .51	0.001
	Control	60	15.50±1.05	
Pcv (%)	Khat chewing	92	49.00±4.25	0.001
	Control	60	46.73±3.53	
RBCs (/mm^3)	Khat chewing	92	5.61±o.61	0.116
	Control	60	5.47±0.39	
WBCs(mm^3)	Khat chewing	92	6.64±1.83	0.776
	Control	60	6.72±1.77	
PLTs(mm^3)	Khat chewing	92	276.91±72.38	0.010
	Control	60	312.26±67.31	
Nutro. %	Khat chewing	92	49.60±12.19	0.682
	Control	60	50.41±11.34	
Lymph. %	Khat chewing	92	39.02±11.26	0.653
	Control	60	39.85±10.77	
Mono. %	Khat chewing	92	6.39±2.20	0.872
	Control	60	6.33±2.10	
Eosn. %	Khat chewing	92	4.75±3.93	0.011

	Control	60	3.26±2.63	
MCV(femolite)	Khat chewing	92	85.23±4.37	0.237
	Control	60	84.41±3.84	
MCH (pg/cell)	Khat chewing	92	28.63±1.42	0.950
	Control	60	28.61±1.16	
MCHc(gm/dl)	Khat chewing	92	32.67±3..37	0.718
	Control	60	32.83±0.61	
RDW %	Khat chewing	92.	14.49±0.79	0.024
	Control	60	14.22±.67	

Table 3-5 CBC Components in study population with Khat chewing (no smoking) and control.

CBC components	Cases	NO.	Mean±std	p-value (t-test)
Hp (gm/dl)	Khat chewing	177	15.63±1 .59	0.561
	Control	60	15.50±1.05	
Pcv (%)	Khat chewing	177	47.02±4.62	0.646
	Control	60	46.73±3.53	
RBCs (/mm^3)	Khat chewing	60	5.40±o.78	0.501
	Control	60	5.47±0.39	
WBCs(mm^3)	Khat chewing	177	6.40±2.04	0.281
	Control	60	6.72±1.77	
PLTs(mm^3)	Khat chewing	177	289.32±80.93	0.049
	Control	60	312.26±67.31	
Nutro. %	Khat chewing	177	49.93±12.44	0.793
	Control	60	50.41±11.34	
Lymph. %	Khat chewing	177	38.83±11.52	0.548
	Control	60	39.85±10.77	
Mono. %	Khat chewing	177	6.70±2.41	0.295
	Control	60	6.33±2.10	

Eosn. %	Khat chewing	177	4.28±3.31	0.32
	Control	60	3.26±2.63	
MCV(femolite)	Khat chewing	177	84.61±4.15	0.751
	Control	60	84.41±3.84	
MCH (pg/cell)	Khat chewing	177	28.61±1.61	0.977
	Control	60	28.61±1.16	
MCHc(gm/dl)	Khat chewing	177	32.83±0..89	0.980
	Control	60	32.83±0.61	
RDW %	Khat chewing	177.	14.32±0.88	0.416
	Control	60	(14.22±.67)	

Table 3- 6 Distribution of case and control groups by duration.

duration groups	Cases	
	Frequency	Percent %
2-12 years	171	63.6 %
13-23	77	28.6%
24-36	21	7.8 %
Total	269	100%

Table 3-7 Distribution of the study population by duration.

Variable	2 – 12 years (N=171)		13 – 23 years (N=77)	24 – 36 years (N=21)	p-value (Anova)
Hp gm/dl	Mean±std	15.73±1.51	16.06±1.62	16.15±2.05	0.111
Pcv %	Mean±std	46.73±3.35	48.23±4.58	48.70±6.20	0.124
RBCs /mm^3	Mean±std	5.46±0.75	5.43±0.68	5.68±0.82	0.526
WBCs($mm^{3)}$	Mean±std	6.40±1.92	6.77±1.98	6.10±2.33	0.352
PLTs(mm^3)	Mean±std	288.3±76.43	284.4±73.29	261.3±105.3	0.037
Nutro. %	Mean±std	49.04±12.67	51.14±11.77	51.33±11.54	0.565
Lymph. %	Mean±std	39.69±11.71	37.66±10.53	36.90±11.97	0.428
Mono. %	Mean±std	6.50±2.29	6.59±2.47	7.28±2.34	0.433

Eosn. %	Mean±std	4.42±3.64	4.51±3.55	4.38±2.71	0.177
MCV (fl)	Mean±std	84.53±3.96	85.49±4.68	84.76±4.57	0.347
MCH(pg/c)	Mean±std	28.50±1.49	28.85±1.71	28.61±1.35	0.312
MCHC(g/dl)	Mean±std	32.83±.73	33.03±1.40	32.85±0.72	0.404
RDW %	Mean±std	14.37±0.877	14.35±0.83	14.49±0.64	0.489

Table 3- 8 CBC Components in male and femal with Khat chewing.

CBC components	Gander	NO.	Mean±std	p-value (t-test)
Hp (gm/dl)	Male	158	15.85±1.47	0.000
	Femal	19	13.81±1.39	
Pcv (%)	Male	158	47.65±4.30	0.000
	Femal	19	41.80±3.94	
RBCs (/mm^3)	Male	158	5.44±0.74	0.000
	Femal	19	4.61± .70	
WBCs($mm^{3)}$	Male	158	6.45±2.05	0.411
	Femal	19	6.04±2.02	
PLTs($mm^{3)}$	Male	158	288.41±78.13	0.667
	Femal	19	296.89±103.59	
Nutro. %	Male	158	49.98±12.29	0.895
	Femal	19	49.57±14.05	
Lymph. %	Male	158	40.15±12.49	0.597
	Femal	19	41.51±12.4	

			1	
Mono. %	Male	158	6.67±2.45	0.639
	Femal	19	6.94±2.12	
Eosn. %	Male	158	4.39±3.41	0.202
	Femal	19	3.36±2.24	
MCV(femolite)	Male	158	84.66±4.16	0.617
	Femal	19	84.15±4.19	
MCH(pg/cell)	Male	158	28.63±1.64	0.492
	Femal	19	28.36±1.38	
MCHc(gm/dl)	Male	158	32.86±0..80	0.139
	Femal	19	32.57±0.60	
RDW %	Male	158	14.37±0.86	0.061
	Femal	19	13.86±0.91	

Table 3- 9 CBC components and quantity (dose) of Khat chewers.

CBC components	quality chewing khat	NO.	Mean±std	p-value (t-test)
Hp (gm/dl)	Small	86	15.66±1.55	0.169
	Large	183	15.95±1.61	
Pcv (%)	Small	86	47.39±4.50	0.448
	Large	183	47.84±4.63	
RBCs (/mm^3)	Small	86	5.52±0.73	0.451
	Large	183	5.44±.74	
WBCs (mm^3)	Small	86	6.30±1.95	0.291
	Large	183	6.57±1.98	
PLTs (mm^3)	Small	86	293.13±69.59	0.248
	Large	183	281.32±81.79	
Nutro. %	Small	86	49.44±13.26	0.728
	Large	183	50.00±11.91	
Lymph. %	Small	86	39.44±11.88	0.592
	Large	183	38.63±11.21	
Mono. %	Small	86	6.72±2.25	0.547
	Large	186	6.53±2.39	

Eosn. %	Small	86	4.02±3.50	0.180
	Large	183	4.64±3.55	
MCV (femolite)	Small	86	84.8±3.97	0.901
	Large	183	84.80±4.35	
MCH (pg/cell)	Small	86	28.51±1.46	0.446
	Large	183	28.66±1.59	
MCHc (gm/dl)	Small	86	32.79±0..76	0.245
	Large	183	32.93±1.06	
RDW %	Small	86	14.36±0.94	0.180
	Large	183	14.39±0.80	

*Large= two bag (more than 250g) *Small = one bag(less or = 250g)

Table 3- 10 Correlation of cell incidences by sex, does and duration.

CBC components		Gander	Does	Duration
HB	Pearson Correlation	-.367**	.107	.103
	Sig. (2-tailed)	.000	.155	.173
	N	237	177	177
PCV	Pearson Correlation	-.375**	.067	.137
	Sig. (2-tailed)	.000	.373	.069
	N	237	177	177
RBCs	Pearson Correlation	-.331**	-.069	-.012
	Sig. (2-tailed)	.000	.363	.870
	N	237	177	177
WBCs	Pearson Correlation	-.054	.054	.092
	Sig. (2-tailed)	.408	.473	. 224
	N	237	177	177
PLTs	Pearson Correlation	.062	-.062	-.069
	Sig. (2-tailed)	.344	.412	.359
	N	237	177	177
Nutrophil	Pearson Correlation	-.053	.082	.140
	Sig. (2-tailed)	.419	.280	.060
	N	237	177	177
Lymphocyt	Pearson	.080	-.055	-.144

	Correlation	.219	.466	.55
	Sig. (2-tailed)	237	177	177
	N			
Monocyt.	PearsonCorrelation	-.020	-.075	.37
	Sig. (2-tailed)	.219	.319	.624
	N	177	177	177
Eosnophil.	Pearson Correlation	-.028	-.002	-.062
	Sig. (2-tailed)	.671	.984	.410
	N	177	177	177
RDW	Pearson Correlation	-.199**	.038	.013
	Sig. (2-tailed)	.002	.615	.858
	N	237	177	177
MCV	Pearson Correlation	-.090	-.012	.036
	Sig. (2-tailed)	.169	.873	.637
	N	237	177	177
MCH	Pearson Correlation	-.092	.046	.003
	Sig. (2-tailed)	.160	.544	.968
	N	237	177	177
MCHC	Pearson Correlation	-.124	.075	-.023
	Sig. (2-tailed)	.56	.319	.766
	N	237	177	177

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Chapter four

Discussion, Conclusion and Recommendation

4.1 Discussion

In Yemen, khat chewing is scheduled for certain times of day within a prescribed setting, and with men and women consuming at separate gatherings (Kennedy, 1987; Meneley, 1996). Hence, in Yemen, as in parts of Ethiopia, both countries with a long history of khat consumption, women have for centuries been able to chew khat in gender segregated groups without fear of being labeled prostitutes (Beckerleg, 2008). The WHO classifies khat as causing psychological but not physical dependence ,with daily consumption causing negative effects on the social and economic life of the user (WHO,2006).The khat effects includes euphoria, excitability, anxiety, irritability, hyperactivity, restlessness and insomnia (Cox and Rampes, 2003). The adverse effects of Qa khat t in the central nervous system, as in other systems, are dose-related (Alem and Shibre, 1997; Cox and Rampes, 2003). Regular khat chewing is associated with elevated mean diastolic blood pressure (Tesfaye et. al, 2008). khat chewing has also been

reported to increase the incidence of acute cerebral infarction. (Mujalli et. al, 2005). Khat chewing during pregnancy has a detrimental effect on the foetus, leading to low birth weight, teratogenic effects and infant mortality (Mwenda et. al, 2003).

The results of the present study showed that, the values of CBC parameters tested in association to khat consumption fell within normal range in the study were. On exception was that platelets found to be reduced significantly compared to value of platelets of non khat chewers. This finding suggests that khat may have an effect on body parameters other than CBC parameters. The studies concerning khat effect CBC were rare accordingly we this work has been conducted.

Narcotic and analgesic effect of khat on CNC has been largely investigated, but its effects on CBC components were poorly studied. The samples included in the study were selected in carefully and systematic fashion using well designed questionnaire and physical examination performed by specialized physician. The aim behind this was to exclude individual with illness and or physical disorders.

Aspirin as preventive therapy against cardio vascular events is widely recommended for healthy people’s wells patients with ischemic heart diseases. A low daily dose of aspirin (75mg) is used for the long-term Prevention of heart attack and stroke. The bleeding time in myocardial infarction patients taking long-term aspirin (100 mg daily) was significantly reduced in khat chewers to 2.3 min compared with 8 minim non- khat chewers taking the

same dose of aspirin (Alkadi et. al, 2008). This finding suggested that a constituent of khat attenuates the ant platelet aggregating proper- ties of aspirin, there by neutralizing the beneficial actions of aspirin. Adrenaline induces aggregation of human platelets which is mediated via alpha 2- adrenoceptors (Broadley, 1996). Support our finding effect platelets and may induced abnormality in coagulation profiles. The effect of khat platelet may not be a direct effect for the reason that pesticides found to reduce platelet count and disturb platelet aggregation but may be effect in the thrombopoietin or receptor of platelet. Accordingly, prolonged exposure to khat could result in psychoneurological disturbances such as neurosis (Hoffman and Al'Absi, 2010). In addition, increased diastolic blood pressure (Getahun, 2010), and vasoconstriction of coronary vasculature were also reported (Zubaid et. al, 2010). More commonly, gastritis (Nencini and Ahmed, 2010), hemorrhoids, and duodenal ulcer had a higher prevalence among khat chewers (Manghi, 2009). Furthermore, Luqman and Danowski reported that liver cirrhosis that was observed among Yemeni khat chewers might be due to khat consumption, but at that time it was not further investigated (Luqman and Danowski, 1976), and hepatotoxicity of khat chewing is still debated in humans (Coton et. al, 2011). In Yemen, many pesticides used randomly for spraying khat trees, so khat chewer consumed khat sprayed by pesticides .This result supported by study (Varol and Gultkin, 2012), who found that effect of pesticides exposure on platelet indices in farm workers

on platelets count ,MPV and PDW, was significantly lower in farm works than those of control (P<0.001).

In addition (Krug and Berudt., 1985) and his coworker have shown that pesticides have effect on platelet aggregation and arachidonic acid metabolism.

Khat in this study has shown no effect in all CBC parameters except platelets and eosinophil this finding supported by the result reported by (El Hadrani et. al, 2000). In this study the result showed that all hematological parameters tested had their values with the normal range. Except platelets which were significantly decreased compared with platelets of control and eosinophil which were significant increased compared with eosinophil of control. This result may shed light on the possible effect of khat on coagulation profile. In the respect khat has been showed to disturb platelets aggregates effects induced by aspirin. And also result may shed light on the possible effect of khat due to don't washing of khat before chewers of khat.

In addition (Alsalahi et. al, 2012) and his coworker as has shown that the effect of khat on male and female rats on Hematological study (RBC,WBC, Plts, HGB, PCV, and MCH) values in blood film of male SD-rats were not different from control (p value >0.05) . Conversely, the (MCHC) in male SD-rats was significantly (p value <0.05), Blood film profile of male SD-rats. (RBC, Plts, HGB, and PCV) in blood film of female SD-rats were not significantly (p value >0.05). Conversely, the value of WBC Compared to control of female SD-rats was significantly (Alsalahi et. al, 2012).

In this study the blood film RBCs,WBCs, and Plts counts in the normal morphology were not affected compared to control normal in all studied subject. Except in some cases (Number 12 cases) there were showed that platelet decreased than normal rang.

In the study the results showed the results CBC Components in male and female with khat chewing are statically different (P value < 0.05) in the parameters tested Hb, PCV, and RBCs. Because different physiological between male and femal,and the female chewers khat less than male . But the other CBC components were in the normal range. In this study showed the results of CBC components (Hb, PCV, and RBCs) in khat chewing in association with smoking was found to have statistically significant effect (P<0.05), on Hb, PCV, RBC count but on the other parameters CBC showed no effect. But the mean of khat chewing in association with smoking on platelets lower than control (276.97±72.25, 289.32±80.93) respectively. Scientific the smoking increased platelets that supported the resulted showed induced platelets in people's chewer's khat.

Based on duration of khat chewing, subjects were, distributed into three categories (2 to 12, 13 to 23 and 24 to 36 years). The obtained results showed no effect of duration on tested CBC parameters expted platlets showe effect in this study.

In addition, (Alsalahi et. al. 2012), and his coworker as has shown that the effect of khat on male and female rats. The (MCV) of female SD-rats was significantly (ANOVA, P < 0.05) different between groups, and post hoc Dunnet test indicated that high

does (HD) was significantly ($P \leq 0.01$) greater than normal control (NC) (by 2%). In addition, the (MCH) in female SD-rats was significantly (ANOVA, $P < 0.05$) different between groups, and post hoc Dunnet test indicated that the mean MCH values of high does(HD) ($P < 0.01$), medium does(MD) ($P \leq 0.01$), and low does(LD) ($P < 0.05$) were significantly higher than that of normal control (NC) (by 8%, 6% and 4%, resp.). Moreover, (MCHC) in female SD-rats was significantly (ANOVA,$P < 0.05$) different between groups and post hoc Dunnet test indicated that the mean MCHC values of both high does and medium does were significantly ($P \leq 0.01$) greater than that of NC (by 4%) (Alsalahi et. al, 2012).

Based on dose of khat chewing, subjects were, distributed into two categories (small and large). The obtained results showed no effect of dose on tested CBC parameters in this study table 3-9.

In this study showed no correlation between khat chewing and the duration, quantity and CBC parameters in this study table 3-10.

Conclusion and Recommendation

4.2 Conclusion

*Khat chewing has no effect on CBC parameters.

*Khat consumption has reduced platelets in the subject of this study.

*Duration of khat chewing has no effect on all CBC parameter tested in this study.

*Dose of khat consumption has no effect on all CBC parameter tested in this saseudy.

*This action of khat was not affected by gender.

*Blood film morphology normal RBC, WBC and Platelets except in some cases showed thrombocytopenia.

4.3 Recommendation

Since khat chewing is widespread in Yemen, the following actions are recommended.

*Increase public awareness of the potential health hazards of khat chewing.

*Support scientific research on khat in different institutions and universities to explore the different effects of khat on public health.

*Doing research about the effected of khat chewing on the coagulation profile, Thrombopoietin hormones and cyclo-oxygenas enzymes.

*Integrate education about khat into the curricula of primary and secondary

Schools.

*Sarvaleence the pesticides using sprayer khat.

* A large studies including large number of peoples chewing khat

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Appendices

Appendix A: Sysmex kx21

Appendix B: Khat farms

Appendix c: peoples khat chewers:

Sudan University Sciences and Technology
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Measurements of Effect Khat Consumption on complete blood count Sana'a City – Yemen

Appendix d: Questionnaire

-Serial number…… ***- Age: ……***

Sex……-Address: ……

Tel. No.: ……

-Location: ***rban***

-marital status: married……, single……,
children……

-Chewing Qat: ***Ye*** ***No***

-Time of chewing Qat session ……Hours per day.

- Frequency Chewing Qat: *:……* ***per week ,,,,,,,,,,,,,,,,,,,,,,per years***

-Quality of chewing Qat : ***small*** ***medium*** ***large***

-Smoking: ***p***

-Safety which used in Chewing Qat: ***No***

- Others:

- CBC (Cell Blood Count):

-Hb…….mg/dL PCV……

-RBCs…… WBCs…….

PLTs……

-N……% -L……% M……% E……%

B……%

-RDW……

-

MCV……MCH……MCHC……

……

Blood Film:

- RBCs……

-WBCs……

Plts……