Sudan University of Science and Technology

Collage of Graduate Studies and scientific research

Measurement of Complete Blood Count Among Fuel Benzene Station Workers in Khartoum State

تعداد الدم الشامل للعاملين في محطات البنزين في ولاية الخرطوم

A thesis Submitted Partial Fulfillment for the Degree of Master in Science in Medical Laboratory Science Hematology and Immunohematology

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الآيـــــــة

قال تعالى

(قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاّ مَا عَلّمْتَنَا إِنّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ)

صدق الله العظيم

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Dedication

First I dedicate this study to the bright light that started from Makkah to cover the world the prophet Mohammed the prayers and peace of Allah to him.

To soul of my mother Amna Noreldaiem

To my father who spent all his life to help me

To my sisters and brothers and friends

To souls of all Sudanese who died by reduction of health care

To any one helped me to complete this research

A special dedication to my great supervisor Dr. Tagwa Yousif

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Abstract

This is a analytical case - control study aimed to measure the change of some hematological parameters in one hundred benzene station workers, people involved in this study were male (18 – 52) years Sudanese benzene station workers in Khartoum state. The study period extended from April to June 2014.

Investigation were performed by using automated cell count (sysmex device) and manual thin blood film. This study assessed the measure hematological parameters in venous blood samples among 100 male as study group and 100 male as control by using sysmex device.

The study reflected that the mean of HB (g/dl) , PCV (%) , RBCs $x10^{12}$ /l. WBCsx 10^{9}, PLTs10^{9}/ L ,MCV(fl), MCH (pg) . MCHc (%) , neutrophils count , lymphocytes count . monocytes count , eosinophils count and basophils count of workers exposed benzene as case group were :

12.6 , 38 , 3.4 , 3.3, 124, 68.7 , 27, 32.1 , 44 , 34 , 13 , 5 , 0.4 , respectively and the result obtained from control group revealed that the mean of HB (g/dl) , PCV (%) , RBCs $x10^{12}$ /l. WBCsx 10^{9}, PLTs10^{9}/ L, MCV(fl), MCH (pg) . MCHC (%) , neutrophils count , lymphocytes count . monocytes count , eosinophils count and basophils count were : 13.3 , 39 , 4.8 , 6.6 , 268 , 77 , 29 , 32.2 , 56 , 36 , 7 , 2 , 0 respectively

The main conclusion derived from this study, there is significant effects in hematological parameters related to exposed to benzene with pancytopenia, eosinophilia, Basophilia and monocytosis and there is association between period of exposure to benzene and panctopenia with increase by increase of working duration.

مستخلص البحث

أجريت هذه الدراسة التحليلية لقياس تأثير البنزين على نسب الدم العام وتحديد التغيرات للعاملين في محطات البنزين الفئة التي تم عليها البحث ذكور أعمارهم بين (18 – 52) عاماً يعلمون في محطات البنزين داخل ولاية الخرطوم فترة هذا البحث امتدت من أبريل إلى يونيو 2014م.

أجريت هذه الدراسة في فورمات خاصة بالبحث وأخذت عينات من الدم من الوريد في حافظات بها مادة مانعة للتجلط وأخذت للمعمل حيث أخذت 100 عينة من العاملين في محطات البنزين و100 عينة قياسية وحللت بواسطة المحلل الاتوماتيكي (جهاز Sysmex).

اشارت نتيجة الدارسة الى ان متوسط قيم الهيموجلوبين (g/dl) ، (%) PCV ، كريات الدم الحمراء ($x10^{12}$/l) ، كريات الدم البيضاء ($x10^{9}$/l) ، الصفائح الدموية ($x10^{9}$/l) ، (FL) MCV ، MCH (pg) ، (%) MCHC ، الخلايا المتعادلة (%) ، الخلايا الليمفاوية (%) ، الخلايا الوحيدة (%) ، الخلايا الحمضية (%) والخلايا القاعدية (%) للعاملين في محطات البنزين كانت 12.6 ، 38 ، 3.4 ، 3.3 ، 124 ، 68.7 ، 27 ، 32.1 ، 44 ، 34 ، 13 ، 5 ، 0.4 على التوالي وان متوسط قيم الهيموجلوبين (g/dl) ، (%) PCV ، كريات الدم الحمراء ($x10^{12}$/l) ، كريات الدم البيضاء ($x10^{9}$/l) ، الصفائح الدموية ($x10^{9}$/l) ، (FL) MCV ، MCH (pg) ، (%) MCHC ، الخلايا المتعادلة (%) ، الخلايا الليمفاوية (%) ، الخلايا الوحيدة (%) ، الخلايا الحمضية (%) والخلايا القاعدية (%) للمجموعة القياسية كانت 13.3 ، 39 ، 4.8 ، 6.6 ، 268 ، 68.7 ، 29 ، 32.2 ، 56 ، 36 ، 7 ، 2 ، 0 على التوالي .

الخاتمة المستنتجة من الأطروحة أنه يوجد تأثير للبنزين على نسب الدم العام حيث أن كل نتائج تحليل الدم الكلي للعاملين بمحطات البنزين تحليله مقارنةً بنتائج تحليل الدم الكلي للعينات القياسية والنتائج أيضاً تشير إلى الارتباط بين فترة التعرض لمادة البنزين وقلة الكريات بزيادة فترة العمل.

Abbreviations.

CBC. Complete Blood Count

CSF. Colony Stimulating Factor

EDT A. Ethylene Di amine Tetra Acetic acid

ESR. Erythrocyte Sedimentation Rate

FBC. Full Blood Count

G-CSF. Granulocyte Colony Stimulating Factor

GM-CSF. Granulocyte Macrophage Colony Stimulating Factor

HB. Haernoglobin

HCT Heamatocrit

HIT. Heparin Induced Thrombocytopenia

HSC. Haematopoietec Stem Cell

MCH. Mean Cell Haemoglobin

MCHC. Mean Cell Haemoglobin Concentration

MCSF. Macrophage Colony Stimulating Factor

MCV. Mean Cell Volume

MPV. Mean Platelet Volume

PCV. Packed Cell Volume

PDGF. Platelet Derived Growth Factor

PMN. Polymorphonuclear

PRP. Platelet Rich Plasma

RBCs. Red Blood Cells

SCF. Stem Cell Factor

TPO. Thrombopoitein

TTP. Thrombocytopenic Purpura

WBCs. White Blood Cell

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Chapter one

Introduction and literature review

1.1 Components of blood:

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells — such as nutrients and oxygen — and transports waste products away from those same cells.

In vertebrates, it is composed of blood cells suspended in a liquid called blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (91% by volume), and contains dissolved proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), platelets and blood cells themselves. The blood cells present in blood are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in vertebrate blood are red blood cells. these contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen) reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellularly dissolved in plasma as bicarbonate ion. (Dicken and Scott , 2004)

Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled. (Dicken and Scott , 2004)

blood performs many important functions within the body including.

a) Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells
b) Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids))
c) Removal of waste such as carbon dioxide, urea, and lactic acid

d) Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies
e) Coagulation, which is one part of the body's self-repair mechanism (blood clotting after an open wound in order to stop bleeding)
f) Messenger functions, including the transport of hormones and the signaling of tissue damage
g) Regulation of body pH
h) Regulation of core body temperature
i) Hydraulic functions . (Dicken and Scott , 2004)

1.1.1 Red blood cells:

Human red blood cells(also referred to as erythrocytes) are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (02) to the body tissues via the blood flow through the circulatory system. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries. These cells' cytoplasm is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the blood's red color . (Erich Sackmann, 1995)

In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles, 2.4 million new erythrocytes are produced per second.3 The cells develop in the bone marrow and circulate for about 100—120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood ce1ls.

Red blood cells are also known as RBCs, red blood corpuscles (an archaic term), haematids, erythroid cells or erythrocytes (from Greek erythros for "red" and kytos for "hollow", with cyte translated as "cell" in modern usage). The capitalized term Red Blood Cells is the proper. (Erich Sackmann, 1995)

1.1.2 White blood cells:

White blood cells (WBCs), or leukocytes (also spelled "leucocytes"), are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of

leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system.5

The number of WBCs in the blood is often an indicator of disease. There are normally between 4 x and 1.1 x 1010 white blood cells in a litre of blood, making up approximately 1% of blood in a healthy adult. An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia. The physical properties of leukocytes, such as volume, conductivity, and granularity, may change due to activation, the presence of immature cells, or the presence of malignant leukocytes in leukemia. (Garther , *et al* ., 2007)

There are several different types of white blood cells. They all have many things in common, but are all distinct in form and function. A major distinguishing feature of some leukocytes is the presence of granules; white blood cells are often characterized as granulocytes or agranulocytes.

a) **Granulocytes (polymorphonuclear leukocytes):**
   leukocytes characterized by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes which primarily act in the digestion of endocytosed particles. There are three types of granulocytes. neutrophils, basophils, and eosinophils, which are named according to their staining properties.
b) **Agranulocytes (mononuclear leucocytes):**
   leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are lysosomes. The cells include lymphocytes, monocytes, and macrophages.
   (Garther , *et al.*, 2007)

1.1.2.1 Neutrophils:

Neutrophils defend against bacterial or fungal infection and other very small inflammatory processes that are usually first responders to microbial infection; their activity and death in large numbers forms pus. They are

commonly referred to as polymorphonuclear (PMN) leukocytes, although technically PMN refers to all granulocytes. They have a multi lobed nucleus which may appear like multiple nuclei, hence the name polymorphonuclear leukocyte. The cytoplasm may look transparent because of fine granules that are pale lilac. Neutrophils are very active in phagocytosing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew their lysosomes used in digesting microbes and die after having phagocytosed a few pathogens. Most common cell seen in acute inflammation, comes in and kill foreign substance. They make up 60-70% of total leukocyte count.The life span of neutrophil is about 8 days.
(wheater, *et al* ., 2002) .

1.1.2.2 Eosinophils:

Eosinophils primarily deal with parasitic infections and an increase in them may indicate such. Eosinophlils are also the predominant inflammatory cells in allergic reactions. The most important causes of eosinophilia include allergies such as asthma, hay fever, and hives; and also parasitic infections. Generally their nucleus is bi-lobed. The cytoplasm is full of granules which assume a characteristic pink-orange color with eosin stain. (wheater, *et al* ., 2002) .

1.1.2.3 Basophils:

basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing inflammation. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules which hide it. They are characterized by their large blue granules. (wheater, *et al* ., 2002) .

1.1.2.4 Lymphocytes:

lymphocytes are much more common in the lymphatic system. Lymphocytes are distinguished having a deeply staining nucleus which may be eccentric in location, and a relatively small amount of cytoplasm. The blood has three types of lymphocytes.

a) B cells. B cells make antibodies that bind to pathogens to enable their destruction. (B cells not only make antibodies that bind to pathogens, but after an attack, some B cells will retain the ability to produce an antibody to serve as a 'memory' system.)
b) T cells.

- CD4+ (helper) T cells co-ordinate the immune response and are important in the defense against intracellular bacteria. In acute HIV infection, these T cells are the main index to identify the individuals immune system activity. Research has shown that CD8+ cells are also another index to identify human's immune activity.
- CD8+ cytotoxic T cells are able to kill virus-infected and tumor cells.
- γδ T cells possess an alternative T cell receptor as opposed to CD4+ and CD8+ cU3 T cells and share characteristics of helper T cells, cytotoxic T cells and natural killer cells.

c) Natural killer cells. Natural killer cells are able to kill cells of the body which are displaying a signal to kill them, as they have been infected by a virus or have become cancerous. (wheater, *et al* ., 2002) .

1.1.2.5 Monocytes:

Monocytes share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an additional role. they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed, or so that an antibody response may be mounted.

Monocytes eventually leave the bloodstream to become tissue macrophages which remove dead cell debris as well as attacking microorganisms. Neither of these can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. They have the kidney shaped nucleus and are typically agranulated. They also possess abundant cytoplasm. (wheater, *et al*., 2002) .

Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and are then known as macrophages. (wheater, *et al* ., 2002) .

1.1.3 Platelets:

Platelets, or thrombocytes are big, regularly-shaped clear cell fragments (i.e. cells that do not have a nucleus containing DNA), 2-3 im in diameter, which are derived from fragmentation of precursor megakaryocytes. The average lifespan of a platelet is normally just 5 to 9 days. Platelets play a fundamental role in hemostasis and are a natural source of growth factors. They circulate in the blood of mammals and are involved in hemostasis, leading to the formation of blood clots.

If the number of platelets is too low, excessive bleeding can occur. However, if the number of platelets is too high, blood clots can form (thrombosis), which may obstruct blood vessels and result in such events as a stroke, myocardial infarction, pulmonary embolism or the blockage of blood vessels to other parts of the body, such as the extremities of the arms or legs. An abnormality or disease of the platelets is called a thrombocytopathy, which could be either a low number of platelets (thrombocytopenia), a decrease in function of platelets (thrombasthenia), or an increase in the number of platelets (thrombocytosis). There are disorders that reduce the number of platelets, such as heparin-induced thrombocytopenia (HIT) or thrombotic thrombocytopenic purpura (TTP) that typically cause thromboses. or clots, instead of bleeding. (Laki , 1972)

Platelets release a multitude of growth factors including Platelet-derived growth factor (PDGF), a potent chemotactic agent, and TGF beta, which stimulates the deposition of extracellular matrix. Both of these growth factors have been shown to play a significant role in the repair and regeneration of connective tissues. Other healing-associated growth factors produced by platelets include basic fibroblast growth factor, insulin-like growth factor 1, platelet-derived epidermal growth factor, and vascular endothelial growth factor. Local application of these factors in increased

concentrations through Platelet-rich plasma (PRP) has been used as an adjunct to wound healing for several decades) . (Laki , 1972) .

1.2 Haematopoiesis:

Haematopoiesis is the formation of blood cellular components. All cellular blood components are derived from haematopoietic stem cell in a healthy adult person, approximately 1011_i 012 new blood cells are produced daily in order to maintain steady state levels in the peripheral circu1ation. (Leif Jonsson , 2008)

Haematopoietic stem cells (HSCs):

Haematopoietec stem cell (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. HSCs are self renewing. when they proliferate, at least some of their daughter cells remain as HSCs, so the pooi of stem cells does not become depleted. The other daughters of HSCs (myeloid and lymphoid progenitor cells), however can each 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. (Leif Jansson , 2008)

Locations of haematopoiesis:

In developing embryos, blood formation occurs in aggregates of blood cells in the yolk sac, called blood islands . As development progresses, blood formation occurs in the spleen .liver and lymph node. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism. However, maturation, activation, and some proliferation of lymphoid cells occurs in secondary lymphoid organs (spleen, thymus, and lymph nodes). In children, haematopoiesis occurs in the marrow of the long bones such as the femur and tibia. In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum. In some vertebrates, haematopoiesis can occur wherever there is a loose stroma of connective tissue and slow blood supply, such as the gut, spleen, kidneys or ovaries. In some cases, the liver, thymus, and spleen may resume their haematopoietic

function, if necessary. This is called extramedullary haematopoiesis. It may cause these organs to increase in size substantially. During fetal development, since bones and thus the bone marrow, develop later, the liver functions as the main haematopoetic organ. Therefore, the liver is enlarged during development. (Leif Jansson , 2008)

Maturation of haematopoiesis:

As a stem cell matures it undergoes changes in gene expression that limit the cell types that it can become and moves it closer to a specific cell type. These changes can often be tracked by monitoring the presence of proteins on the surface of the cell. Each successive change moves the cell closer to the final cell type and further limits its potential to become a different cell type . (Leif Jansson , 2008)

Determination of haematopoiesis:

Cell determination appears to be dictated by the location of differentiation .For instance , the thymus provides an ideal environment for thymocytes to differentiate into a variety of different functional T cells. For the stem cells and other undifferentiated blood cells in the bone marrow , the determination is generally explained by the determinism theory of haematopoiesis, saying that colony stimulating factors and other factors of the haematopoietic microenvironment determine the cells to follow a certain path of cell differentiation. This is the classical way of scribing haematopoiesis. In fact, however, it is not really true. The ability of the bone marrow to regulate the quantity of different cell types to be produced is more accurately explained by a chastic theory. Undifferentiated blood cells are determined to specific cell types by randomness. The haematopoietic microenvironment prevails upon some of the cells to survive and some , on the other hand, to perform apoptosis and die. By regulating this balance between different cell types, the bone marrow can alter the quantity of different cells to ultimately be produced . (Leif Jansson , 2008)

Haematopoietic growth factors:

Red and white blood cell production is regulated with great precision in healthy humans, and the production of granulocytes is rapidly increased during infection. The proliferation and self-renewal of these cells depend on stem cell factor (SCF). glycoprotein growth factors regulate the proliferation and maturation of the cells that enter the blood from the marrow, and cause cells in one or more committed cell lines to proliferate and mature. Three more factors that stimulate the production of committed stem cells are called colony-stimulating factors (CSFs) and include granulocyte-macrophage CSF (GM-CSF), granulocyte CSF (G-CSF) and macrophage CSF (M-CSF). These stimulate much granulocyte nation and are active on either progenitor cells or end product cells .Erythropoietin is required a myeloid progenitor cell to become an erythrocyte. On the other hand, thrombopoietin makes myeloid progenitor cells differentiate to megakaryocytes (thrombocyte-forming cells) . (Leif Jansson , 2008)

Transcription factors:

Growth factors initiate signal transduction pathways, altering transcription factors, that, in turn activate genes that determine the differentiation of blood cells. the early committed progenitors express low levels of transcription factors that may commit them to discrete cell lineages. Which cell lineage is selected for differentiation may depend both on chance and on the external signals received by progenitor cells. Several transcription factors have been isolated that regulate differentiation along the major cell lineages. For instance, PU. 1 commits cells to the myeloid lineage whereas GATA-1 has an essential role in erythropoietic and megakaryocytic differentiation. The Ikaros, Aiolos and Helios transcription factors play a major role in lymphoid development. (Leif Jansson , 2008)

1.2.1 Erythropoiesis:

Erythropoiesis is the process by which red blood cells (erythrocytes) are produced. It is stimulated by decreased O_2 delivery to the kidneys, which then secrete the hormone erythropoietin .This activates increased erthropoiesis in the hemopoietic tissues .By the third or fourth month,

erythropoiesis moves to the spleen and liver. After seven months, erythropoiesis occurs in the bone marrow. However, in humans with certain diseases, erythropoiesis also occurs outside the bone marrow, within the spleen or liver. This is termed extramedullary erythropoiesis .The bone marrow of essentially all bones produces RBCs until a person is five year old .The tibia and femur cease to be important sites of hematopoiesis by about age 25; the vertebrae, sternum, pelvis and ribs, and cranial bones continue to produce red blood cells throughout life . (Bhushan, etal. , 2010)

In the process of red blood cell maturation, a cell undergoes a series of differentiations. The following stages 1-7 of development all occur within the bone marrow.

1. hemocytoblast a pluripotent hematopoietic stem cell
2. . Common Myloid Progenitor multipotent stem cell.
3. unipotent stem cell.
4. pronormoblast also commonly called proerythroblast or rubriblast.
5. basophilic normoblastlearly normoblast also commonly called erythroblast.
6. polychromatophilic nomoblast/intermediate normoblast .
7. orthochromatic normoblastllate normoblast - Nucleus is Expelled before becoming a reticulocyte.
8. reticulocyte.

the cell is released from the bone marrow after stage 7, and so of circulating red blood cells thare 1% - reticulocytes. After 1-2 days these ultimately become “erytbrocytes” or mature red blood cells. these stages correspond to specific appearances of the cell when stained with Wright’s stain and examined by light microscopy, but correspond to other biochemical changes. (Bhushan, *et al.* , 2010)

the process of maturation a basophilic pronormoblast is converted from a cell with a large nucleus and a volume of 900 fL to an enucleated disc with a volume of 95 fL. By the reticulocyte stage, the cell has extruded its nucleus, but is still capable of producing hemoglobin. essentially important for the maturation of RBC’S are two vitamins B-12 and folic acid. Lack of any one

of these causes maturation failure in the process of erythropoiesis. (Bhushan, *et al.* , 2010)

the following characteristics can be seen changing in the erythrocytes when they are maturing.

a) They show a reduction in the cell size;
b) The cytoplasmic matrix increases in amount;
c) Staining reaction of the cytoplasm changes from basophilic to acidophilic (this is because of the decrease in the amount of RNA and DNA);
d) Initially the nucleus was large in size and contained open chromatin. But with the maturation of RBC's the size of the nucleus decreases and finally disappears with the condensation of the chromatin material. (Bhushan, *et al.* , 2010)

Erythropoietin helps regulate the process of erythropoiesis so that, in non-disease states, the production of red blood cells is equal to the destruction of red blood cells and the red blood cell number is sufficient to sustain adequate tissue oxygen levels but not so high as to cause sludging,thrombosis, or stroke. Erythropoietin is produced in the kidney and liver in response to low oxygen levels. In addition, erythropoietin is bound by circulating red blood cells; low circulating numbers lead to a relatively high level of unbound erythropoietin, which stimulates production in the bone marrow. The peptide hormone hepcidin which produced by liver may play a role in the regulation of hemoglobin production, and thus affect erytbropoiesis. Hepcidin controls iron absorption in the gastrointestinal tract and iron release from reticuloendothelial tissue. Iron must absorption from macrophages in the bone marrow to be incorporated into the heme group of hemoglobin in erythrocytes. There are colony forming units that the cells follow during their formation. These cells are referred to as the committed cells including the granulocyte monocyte colony forming units. (Bhushan, *et al.* , 2010)

1.2.2 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 (megakaryocytes), and the two main groups of WBCs, myelocytes and lymphocytes. (USA National library of Medicine , 2014)

Lymphopoiesis refers to the generation of lymphocytes, one of the five different types of white blood cells (WBC), and is also more formally called lymphoid hematopoiesis." (USA National library of Medicine , 2014)

Myelopoiesis is the regulated formation of myeloid cells, including eosinophilic granulocytes. basophilic granulocytes, neutrophilic granulocytes, and monocytes. In hematology, myelopoiesis is the production of blood cells in the bone marrow. (USA National library of Medicine , 2014)

The myeloid progenitor can differentiate in the bone marrow into granulocytes, macrophages (mature monocytes), mast cells (whose blood-borne progenitor is not well defined), and dendritic cells of the innate immune system. The granulocytes, also called polymorphonuclear leukocvtes because of their oddly shaped nuclei, give rise to three short lived cell types including eosinophils basophils, and neutrophils. A granulocyte differentiates into a distinct cell type by a process called granulopoiesis. In this process it first transforms from a common myeloblast (myeloid progenitor) to a common promyelocyte. This promyelocyte gives rise to a unique myelocyte that for the first time can be classified as a eosinophil, basophil, or neutrophil progenitor based on the histo1oical staining affinity (eosinophilic, basophilic, or neutral granules). The unique myelocvte next differentiates into a metamyelocyte and then a band cell, with a "c" shaped nucleus, before becoming a mature eosinophil, basophil, or neutrophil.

Macrophages come from monoblast progenitors that differentriate into promonocytes, which mature into monocytes. Monocvtes eventually enter the tissues and become macrophages. (USA National library of Medicine , 2014)

1.2.3 Megakaryopoiesis:

The process of megakaryopoiesis and platelet production is complex, with the potential for regulation at multiple stages. Megakaryocytes are derived from the hematopoietic stem cell through successive lineage commitment steps, and they undergo a unique maturation process that includes polyploidization, development of an extensive internal demarcation membrane system. and finally formation of pro-platelet processes. Platelets are shed from these processes into vascular sinusoids within the bone marrow. Megakaryocyte differentiation is regulated both positively and negatively by transcription factors and cytokine signaling. Thrombopoietin (TPO) is the most important hematopoietic cytokine for platelet production. Clinically, acquired and inherited mutations affecting megakaryocytic transcription factors and thrombopoietin signaling have been identified in disorders of thrombocytopenia and thrombocytosis. (SCHULMAN, *et al*. ,1960)

1.3 Benzene:

Benzene is a chemical that is often used in manufacturing. In its most common form, benzene is a liquid that is clear, slightly sweet smelling, and highly combustible. Benzene is frequently used in manufacturing rubber, paint, plastics, resins, drugs, pesticides, synthetics, and other products. It is also present in gasoline and tobacco smoke. (Verschueren, 1983)

A known carcinogen, benzene can be harmful to those exposed to it over an extended period of time. Benzene exposure is most likely to occur among workers in facilities that use the chemical in their products. In addition, benzene can enter the environment through spills, accidental releases. volcanic eruptions, and forest fires. It evaporates quickly in air and is partially soluble in water .Benzene may sometimes be referred to as.

benzol 90, pyrobenzol, phene, coal naphtha, and polystream. (Verschueren, 1983).

1.3.1 physical properities .

Benzene is clear.non –corrosive and highly fammable liquid.which is colorless and has strong sweet odour with relative high milting poient , (Langley , 2005)

1.3.2 chemical properties .

Benzene is an organic chemical compound with the molecular formula C_6H_6 . Its molecule is composed of 6 carbon atoms joined in a ring . with I hydrogen atom attached to each carbon atom . because its molecules contain only carbon and hydrogen atoms , benzene is classed as a hydrocarbon . (Langley , 2005) .

Benzene is a colorless and highly flammable liquid with a sweet smell . because it has a high octane number , it is an important component of gasoline . comprising a few percent of its mass . (Langley,2005) .

1.3.3 benzene structure .

The carbons are arranged in a hexagon and he suggested alternating double and single bonds between them . each carbon atom has a hydrogen attached to it . this diagram is often simplified by leaving out all the carbon and hydrogen atoms (Langley , 2005) .

1.3.4 metabolism of benzene .

Qualitatively , the metabolism and elimination of benzene appear to be similar in humans and laboratory animals . benzene is metabolized mainly in the liver but also in other tissuer , such as the bone marrow . (Langley, 2005) .

The metabolites responsible for benzene toxicity are not yet fully understood . the key toxic metabolites for cytotoxicity and the induction of leukaemia are thought to be benzoquinone , benzene oxide and muconaldehybe , the genotoxic activity of benzene metablolites is thought to be clastogenic

(causing chromosomal damage) rather than acting through point mutations . benzoquinone and muconaldehyde are both reactive , bipolar compounds known to be clastogenic and the pathways leading to their formation are favoured at low concentrations in both mice and humans . (Langley , 2005)

Benzene is formed from both natural processes and human activities. Natural sources of benzene include volcanoes and forest fires. Benzene is also a natural part of crude oil, gasoline, and cigarette smoke. Benzene is widely used in the United States . It ranks in the top 20 chemicals for production volume. Some industries use benzene to make other chemicals that are used to make plastics, resins. and nylon and synthetic fibers. Benzene is also used to make some types of lubricants, rubbers. dyes, detergents, drugs, and pesticides. Benzene can be present in the soil, air, or water. Because benzene can evaporate, automobile exhaust, manufacturing pollution, and other sources can contribute to the presence of benzene in the air. If benzene is spilled, it is able to partially dissolve in water and can seep into the surrounding soil. Humans most often come into contact with benzene either by breathing it in, drinking contaminated water, or through skin absorption.'4Also Outdoor air contains low levels of benzene from tobacco smoke, gas stations, motor vehicle exhaust, and industrial emissions. Indoor air generally contains levels of benzene higher than those in outdoor air. The benzene in indoor air comes from products that contain benzene such as glues, paints, furniture wax, and detergents. The air around hazardous waste sites or gas stations can contain higher levels of benzene than in other areas. Benzene leaks from underground storage tanks or from hazardous waste sites containing benzene can contaminate well water. People working in industries that make or use benzene may be exposed to the highest levels of it. A major source of benzene exposure is tobacco smoke. (Verschueren, 1983)

Benzene can causing cells not to work correctly. For example, it can cause bone marrow not to produce enough red blood cells, which can lead to anemia. Also, it can damage the immune system by changing blood levels of antibodies and causing the loss of white blood cells. The seriousness of poisoning caused by benzene depends on the amount, route, and length of

time of exposure, as well as the age and preexisting medical condition of the exposed person. (Verschueren, 1983)

People who breathe in high levels of benzene may develop the following signs and symptoms within minutes to several hours.

a) Drowsiness
b) Dizziness
c) Rapid or irregular heartbeat
d) Headaches
e) Tremors
f) Confusion
g) Unconsciousness
h) Death (at very high levels) (Verschueren, 1983)

Eating foods or drinking beverages containing high levels of benzene can cause the following symptoms within minutes to several hours.

a) Vomiting
b) Irritation of the stomach
c) Dizziness
d) Sleepiness
e) Convulsions
f) Rapid or irregular heartbeat
g) Death (at very high levels)

If a person vomits because of swallowing foods or beverages containing benzene, the vomit could be sucked into the lungs and cause breathing problems and coughing. Direct exposure of the eyes, skin, or lungs to benzene can cause tissue injury and irritation .Showing these signs and symptoms does not necessarily mean that a person has been exposed to benzene. (Verschueren, 1983)

Benzene exposure is most dangerous when it occurs over a long period of time or when the concentration of benzene to which a person is exposed is very high. Contact with low to moderate levels of benzene for a short time can cause headaches, vomiting, disorientation, shakiness, elevated heart rate,

and loss of consciousness. Very high levels of exposure can be fatal. People who work with benzene or who are exposed to it over a long period of time are at the highest risk for developing benzene-related illnesses, which range from anemia to cancer.'4 The major effect of benzene from long-term exposure is on the blood. (Long-term exposure means exposure of a year or more.) Benzene causes harmful effects on the bone marrow and can cause a decrease in red blood cells, leading to anemia. It can also cause excessive bleeding and can affect the immune system, increasing the chance for infection. (Verschueren, 1983)

Benzene usually enters the body in one of three ways. skin contact, consumption of tainted water or food, or inhalation. Inside the body, benzene enters the bloodstream and is carried into the bone marrow and fatty tissues. Eventually it passes through the liver, where it is broken down. As a result, harmful metabolites are formed. Some of the health problems caused by benzene exposure are due to the presence of metabolites in the body. (Verschueren, 1983)

1.3.5 Carcinogenicity of benzene:

Benzene is carcinogenic in rats and mice after oral and inhalation exposure, producing malignant tumors at many sites. In a study by the National Toxicology Program, it was administered by gavage in corn oil *5* clays per week for 103 weeks at doses of 0, *5*, 100, or 200 mg/kg of body weight to F344/N rats and *0,25, 50,* or 100 mg/kg of body weight to B6C3F1 mice. Compound-related non-neoplastic or neoplastic effects on the haematopoietic system, Zymbal gland, forestomach, and adrenal gland were seen in both sexes of both species. In addition, the oral cavity was affected in rats, and the lung, liver harderian gland, preputial gland, ovary, and mammary gland in mice. (Wallace , *et al*. ,1987)

1.3.6 Reproductive Effects:

Animal studies show that inhaling benzene vapors can damage reproductive organs and cause infertility. Exposure to benzene in workplaces has caused menstrual variations. (Merian and Zander ., 1982)

1.3.7 Disease that associated with benzene exposure:

In this study the scientists examined blood samples from "10" workers whose exposed to benzene in shoe factory in Italy. Most of these workers show immune suppressive effects and few of them show features of preleukemic phase in their blood. (Baker, , *et al.* ,1981)

1.3.8 Benzene treatment increase production of nitric oxide:

Nitric oxide is a short-lived reactive mediator that inhibits bone marrow cell proliferation induced by 'Gm-CSF'.

Nitric oxide has also been found to inhibit 'M-CSF' induced growth of mouse bone marrow cells and treatment of mice with benzene causes bone marrow depression and leukemogenesis by impaired stromal cells functions, increased number of phagocytes and produce increased amount of tumor necrosis factor and 'Inter leukin-1' and 'H_2O_2'. and Increase production of nitric oxide by bone marrow cells, this because.

- Increase sensitivity to the growth inhibitory effect of combination of 'M-CSF and LPS'.

- Increase expression of'MRNA' for the inducible form of nitric oxidesynthase 'INOS'. They discover that 'NOSI', NG monomethyle 'L-arginine' (L-NMA) also hematotoxic causing decreased bone marrow proliferation. This suggests that in vivo action of 'L-NMA' is not limited to inhibition of 'NOS's'. (Punjbi, , *et al.* ,. 1994)
-

1.3.9 Development of leukemia in pancytopenic patient due to benzene:

In a follow up study done in Turkey on '44" pancytopenic patient due to chronic exposure to benzenes indicate that most patients died from complication of a plastic anemia or from leukemia and there is relationship between age, duration of exposure and pancytopenia outcome. There is a relationship between the level of HbF, HbA and the outcome of pancytopenia in which present only in leukemic states so very low level of HbA2 with or without high level of HbF during pancytopenic state may be a sign of leukemia. (Wallace , *et al.* ,1987,)

1.4 Complete blood count:

A complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) o blood panel, is a test panel requested by a doctor or other medical professional that gives

information about the cells in a patient's blood. It gives important information about the kinds and the numbers of cells in the blood especially red blood cells, white blood cells and platelets. It helps health professional check any symptoms, such as weakness, fatigue , or bruising, you may have, also helps to diagnose conditions such as anaemia. The CBC is also, used to diagnose and manage numerous diseases. The results can reflect problems with fluid volume (such as dehydration) or loss of blood. It can show abnormalities in the production, life span, and rate of destruction of blood cells. It can reflect acute or chronic infection allergies, and problem with clotting . (verso, 1962)

Laboratory test used for CBC:

1.4.1 Automated blood count:

The blood is well mixed (though not shaken) and placed on a rack in the analyzer. This instrument has many different components to analyze different elements in the blood. The cell counting component counts the numbers and types of different cells within the blood. The results are printed out or sent to a computer for review.

Blood counting machines aspirate a very small amount of the specimen through narrow tubing. Within this tubing, there are sensors that count the number of cells going through it, and can identify the type of cell; this is flow cytometry. The two main sensors used are light detectors, and electrical impedance. One way the instrument can tell what type of blood cell is present is by size. Other instruments measure different characteristics of the cells to categorize them. Because an automated cell counter samples and counts so many cells, the results are very precise. However, certain abnormal cells in the blood may be identified incorrectly, and require

manual review of the instrument's results and identifying any abnormal cells the instrument could not categorize.

In addition to counting, measuring and analyzing red blood cells, white blood cells and platelets, automated hematology analyzers also measure the amount of hemoglobin in the blood and within each red blood cell. This information can be very helpful to a physician who, for example, is trying to identify the cause of a patient's anemia. If the red cells are smaller or larger than normal, or if there's a lot of variation in the size of the red cells, this data can help guide the direction of further testing and expedite the diagnostic process so patients can get the treatment they need quickly.

Automated blood counting machines include the Medonic M Series, Beckman Coulter LH series, Sysmex XE-2100, Siemens ADVIA 120 & 2120, the Abbott Cell-Dyn series, and the Mindray BC series . (Buttarello, *et al* ., 2008)

1.4.2 Manual blood count:

Counting chambers that hold a specified volume of diluted blood (as there are far too many cells if it is not diluted) are used to calculate the number of red and white cells per litre of blood.

To identify the numbers of different white cells, a blood film is made, and a large number of white cells (at least 100) are counted. This gives the percentage of cells that are of each type. By multiplying the percentage with the total number of whit& blood cells, the absolute number of each type of white cell can be obtained.

The advantage of manual counting is that automated analysers are not reliable at counting abnormal cells. That is, cells that are not present in normal patients and are only seen in the peripheral blood with certain haematological conditions. Manual counting is subject to sampling error because so few cells are counted compared with automated analysis.

Medical technicians examine blood film via a microscope for 30% of CBCs, not only to fmd abnormal white cells, but also because variation in

the shape of red cells is an important diagnostic tool. Although automated analysers give fast, reliable result regarding how many red cells, the average size of the red cell, and the variation in size of the red cells, they don't detect cells' shapes. Also, home normal patients' platelets will clump in EDTA anticoagulated blood, which causes automatic analysers to give a falsely low platelet count. The technician viewing the slide in these cases will see clumps of platelets and can estimate if there are low, normal, or high numbers of platelets. (David and Dugdale , 2012)

1.4.3. Haemoglobin (HB):

Heanioglobin is protein molecule within red blood cells that carries oxygen and red colour. It composed of two parts, heam part (iron + protoporphyrin), globin part(four polypeptides chains). Normal range for heamoglobin is different according to age and gender and is approximately 13- 18 grams per deciliter for men and 12-16 for women (international units 8.1-11.2 mill moles/liter for men, 7.4-9.9 for women). (David and Dugdale , 2012)

1.4.4 White blood cells and differential count:

A phiebotomist collects the specimen, in this case blood is drawn in a test tube containing an anticoagulant(EDTA, some times citrate) to stop it from clotting ,and transported to a medical technologist in the 1aboratory. (David and Dugdale , 2012)

1.4.5 Heamatocrit (PCV):

In the CBC , we determine the number of red blood cell in several different ways. The quickest easiest is called the heamatocrit , is also referred to as the packed cell volume (PCV) . A blood sample is placed in a tiny glass tube spun in a centrifuge. This device spins the tube round and round at several thousand revolutions per minute . The cells are heavier than the plasma and are compacted at one end of the tube . After the tube is spun , it is examined and the PCV is determined as the percentage of the cellular portion relative to the total amount of blood in the tube (i.e., remainder being the plasma). If the PCV is low, there are fewer red cells in the body than we

would expect, this condition is referred to as anaemia. Aneamias are further classified as either regenerative or non regenerative .In the former, even though the number of the red blood cells is lower than normal, the body is responding by releasing new reticulocytes into the circulation. In the non regenerative aneamia , there are no or very few immature RBCs in the sample and the body continues to lose red blood cells , but no new ones are produced . A non regenerative anaemia is very , very serious and will quickly become life — threatening.

When the PCV is greater than 55, it is said to elevated . This is seen in dehydrated animals as their blood is becoming more concentrated . It is noted in other conditions, such as some cases of the shock, response to high altitude (the air is thinner, therefore there is less oxygen, so more RBCs are put into circulation), diseases of the lungs , etc Remember that, any thing that decreases the amount of oxygen reaching the tissues of the body will cause higher numbers of RBCs to be found in the CBC Normal range for heamatocrit is different between the sexes and is approximately 45-52% for men and 37-48% for women . (David and Dugdale , 2012)

1.4.6 Red cell count:

The number of red blood cells in a volume of blood . Normal range varies slightly between laboratories but is generally between 4.2-5.9 million cells/cmm. This can also be referred to as the erythrocyte count and can be expressed in international units as 4.2-5.9 x 1012 cells per liter.09’ 21) (David and Dugdale , 2012)

1.4.7 Platelets coun:

A platelet count is a test to measure how many platelets you have in your blood. Platelets help the blood clot. They are smaller than red or white blood cells .The number of platelets in your blood can be affected by many diseases. Platelets may be counted to monitor or diagnose diseases, or identify the cause of excess bleeding. Normal Results 150,000 to 400,000 platelets per microliter. (David and Dugdale , 2012)

1.4.8 Mean cell volume(MCV):

The average volume of a red cell. This is a calculated value derived from the heamatocriy and red cell count . Normal range is 86-98 femtoliters. (David and Dugdale , 2012)

1.4.9 Mean cell heamoglobin(MCH):

The averege amount of heamoglobin in the average red cell. This is a calculated value derived from the measurement of heamoglobin and the red cell count. Normal range is 27-3 2 pieograms. (David and Dugdale , 2012)

1.4.10 Mean cell heamoglobin concentration(MCHC):

The average concentration of heamoglobin in a given volume of red cells . This is a calculated volume derived from the heamoglobin measurement and the heamatocrit . Normal range is 32- 36%. (David and Dugdale , 2012)

1.5. Previous study.

Study done in turkey to measure Hematological effect on chronic benzene poisoning in 217 workers:

- A hematological study consisting of the determination of RBC, WBCs PCV, platelets and differential counts was carried out, together with bone marrow puncture and hemoglobin analysis in appropriate case, on a control population of 100 normal people and on 217 male labourers, 95% of whom worked with solvent containing benzene in small shop manufacturing shoes under unhygienic conditions, the concentration of benzene in work places ranged between 30 and 210 p.p.m and period pf exposure between 3 months and 17 years.
- In 51 of the 217 workers (23.50%) hematological abnormalities attributable to chronic benzene poisoning wre detected. Thus, leucopenia was present in 9.70%, Thrombocytopenia in 4.6%, pancytopenia in 2.76%, acquired pseudo-pelger-Huet anomaly in 0.46%, ly mphocytosis in 0.46%, giant platelets in 0.46%, eosinophilia in 2.30%, basophilia in 0.46%.
- Acquired pseudo-pleger-Huet anomaly was detected in a workers with heterozygous betathalassacmia (Hb A2 4.1% and Hb. F 8.7%).

On the other hand, in 33.1% of workers the Hemoglobin less than 12g/dl and in 32.7% the PCV was less than 40%, with the MCV ranging between 86 and 96 Mm3, in non of them did MCV exceed 100 Mm3.

- Thus it is concluded that benzene exerts harmful effect, primary on the leucocytes with eosinophilia and Basophilia, Secondary on the

platelets causing Thrombocytopenia and finally on all three series giving rise to pancytopenia. (Akosy ,1969).

Another study conducted in Nigeria in 2006 titled as Effect of Petroleum Products Inhalation on Some Haematological Indices of Fuel Attendants in Calabar Metropolis

Nigeria showed that, A total of 400 subjects (200 males and 200 females) aged between 18-30 years participated. Each gender was further categorized into two groups of 100 each for control and test, respectively. The test group was again subdivided into test 1 (Ti) and test 2 (T2) in both sexes. Ti subjects were exposed to petroleum fumes for two years and below while T2 subjects were exposed for more than two years. Samples of blood were collected daily and subjected to haematological analysis. The results obtained showed that in males and females, red blood cell counts (106 /mm3) was significantly ($P<0.001$) decreased in Ti (4.4 ± 0.13) and T2 (3.85 ± 0.07) compared to control (4.76 ± 0.01). There was a significant decrease ($P<0.Oi$) in white blood cell counts, haematocrit, haemoglobin concentration, mean corpusciular haemoglobin concentration (MCHC) in both sexes of test groups when compared with control. There was also a significant ($P<0.00i$) decrease in mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) in test 2 males compared with control. Most subjects exposed for longer than two years (T2) had significantly ($P<0.001$) lower values of red blood cell count, haemoglobin concentration and haematocrit than those exposed for less than two years. The odds/odds ratio that a subject would become anaemic progressively rose from less than 1 in the control to greater than 1 or infinity on exposure to petroleum fumes. These results indicate that the petroleum fumes cause a reduction in

haematological indices which worsens with prolonged exposure. (A.M.OKORD, *et al* ., 2006)

1.6 Rationale:

Benzene can cause bone marrow not to produce enough red blood cells, which can lead to anemia. Also, it can damage the immune system by changing blood levels of antibodies and causing the loss of white blood cells. The seriousness of poisoning caused by benzene depends on the amount, route, and length of time of exposure, as well as the age and preexisting medical condition of the exposed person.

There are no enough published paper carried out in Sudan. That shows effects of benzene on blood parameters among petroleum station workers, there for I done this study to explain this effects.

This study done to determine the effect of benzene on blood parameters and express their harmful on health of workers to do suitable protection.

1.7 Objectives:

1.7.1. General objectives.

To measure the complete blood count among Benzene station workers.

1.7.2. Specific objectives.

A) To detect relation between duration of exposure to benzene and their effects on blood parameters.
B) To measure the effects of benzene on RBCs parameters (count, Hb estimation, RBCs indices)
C) To measure the effects of benzene on WBCs parameters (total count, differential leukocyte count)
D) To measure the effects of benzene on platelets number

Chapter Two

Materials and Methods

2.1 Study Design:

This analytical case –control study conducted in petroleum station in Khartoum state, this study aimed to measure complete blood count among benzene station workers.

2.2 Study period.

Approximately 3 months, from April to June 2014.

2.3 Study population:

Petroleum station workers.

2.3.1 Inclusion criteria.

All Benzene station workers available at the time of this study.

2.3.2 Exclusion criteria.

Non Benzene station workers and worker with known hematological disorder before exposed to benzene products.

2.4 Sample size.

There are 200 samples were including in this study, 100 samples from benzene station workers and 100 samples from non benzene station workers as internal quality control.

2.5 Sample and sampling procedure:

A venous blood sample was collected by disposable plastic syringe from anticubital vein, after cleaning with 70% alcohol in 2.5 ml EDTA.

- Gentle and adequate mixing of sample was applied by mixture to avoid hemolysis, clot or platelet aggregation. (Monica, 2000)

2.6 Measure of CBC by automated autowated cell count (sysmex device):

EDTA blood samples were analyzed for CBC by sysmex automated hematological analyzer.

2.7 Principle of automated autowated cell count (sysmex device):

The blood cells are counted in systems based on either Aperture impedance (voltage – pulse) or light – scattering technology (electro – optical counting).

It began as simple counting instrument and has developed into very sophisticated analyzers capable of producing simultaneous count of all the three blood cells type, hemoglobin values and red cell indices.

After that the instrument has been developed that can produce data related to variation in cell size, as well as being able to provide data on differential white cell count. The most commonly used type in hematology laboratories is the impedance cell counters. (Suttor, 1995)

2.8 Method:

2.8.1 Automated technique (principle & method):

A blood cell counters sysmex KX-21 was used. The whole blood mode sample without pre-dilution. The sample number was entered before each sample.

A well mixed anticoagulated sample was set to the sample probe, and the start switch was pressed till the aspirating process was finished. (Volume aspirated approx. 50 ML).

The sample was removed straight down and the sample probe was automatically cleaned.

The aspirated sample was then automatically suspended into the different detector blocks and different parameter was measured.

The results of parameters were then viewed on the screen and subsequently printed out. (Buttarello, etal ., 2008)

2.8.2 Manual technique (principle & methods):

By assessment from well-spread and stained blood film.

- Films were left to dry.
- After drying well they were covered by leishman stain for 3min.
- Then double volume of leishman buffer (PH 6.8) was added and left for further 7 min. the slides were washed under tap water.
- The films were then wiped clean on the other side, and left to dry.

- Lastly the well-spread, stained and dried blood films were examined microscopically using X40 objectives lens and the WBCs differential count was done in the area where the red cells are evenly distributed after counting 100 WBCs.
- Then the percentage and absolute value was calculated. (cheesbrough , 2000)

2.9 Data analysis.

Results will be processed and formulated into tables using Microsoft Excel computer programmed.

2.10 Ethical consideration:

It was considered that all information obtained from participants was kept as highly confidential data and specimens results were not permitted.

The participators were provided with information about the study and any risk which may be raised especially when the collection technique was applied.

Chapter Three

3. Result.

During this study 200 venous blood samples, I am analyzed at Khartoum teaching hospital for hematological parameters of TWBCs and differential count, RBCs count, HGB, HCT, MCV, MCH, MCHC, and platelets count, were done using automated method (sysmex KX).

- Table (3-1): Distribution of study population according to Age, the age ranged from 18 – 52 years.
- Table (3-2): Frequency of working duration among case group per years which ranged from 1 – 15 years with majority working duration from 1 – 5 years.
- Table (3-3): Hematological parameters among benzene workers as case group and control and the result as followed:

- Hemoglobin concentration is reduce in workers in benzene station when compare with control.
- Red cell count is reduce in workers in benzene station when compare with control.
- Total white blood cell count is reduce in workers in benzene station when compare with control.
- Mean cell volume is low in workers exposed to Benzene station when compare with control.
- Mean cell hemoglobin is low in workers exposed to Benzene station when compare with control.
- Mean cell hemoglobin concentration is low in workers exposed to Benzene

- Platelet count is reducing in workers in benzene station when compared with control.
- Eosiophilia, Basophilia and monocytosis. Show in workers in benzene station.

Table (3-4): The relation between hematological parameters of workers in benzene station and duration of work.

This table shows the pancytopenia, Eosiophilia, Basophilia and monocytosis are increased by increasing of working duration.

Table (3.1): Distribution of study population according to Age:

ITEM NAME	Number	Minimum (years)	Maximum (years)	Age mean
Case	100	18	52	30
Control	100	19	56	29

Table (3.2): Frequency of working duration among case group per years

Working duration Per year	Frequency
1-5	55
6-10	30
11-15	15
Total	100

Table (3.3): Hematological parameters among benzene workers as case group and control

Parameters	Population	Mean ± SD	P value
TWBCs $x10^9$/L	Case	3.3 ± 0.6	0.003
	Control	6.6 ± 1.5	
RBCs $x10^{12}$/L	Case	3.4 ± 0.4	0.001
	Control	4.8 ± 0.6	
HB(g/dl)	Case	12.6 ± 1.0	0.004
	Control	13.3 ± 1.1	
HCT(%)	Case	38 ± 2.9	0.003
	Control	39 ± 3.6	
MCV(FL)	Case	77 ± 8.9	0.004
	Control	85 ± 6.5	
MCH(Pg)	Case	27 ± 4.6	0.002
	Control	29 ± 1.5	
MCHC(%)	Case	32.1 ± 1.9	0.000
	Control	32.2 ± 1.5	
PLTs $x10^9$/L	Case	124 ± 30.4	0.002
	Control	268 ± 72	
N(%)	Case	44 ± 10.1	0.000
	Control	56 ± 12.4	
L(%)	Case	34 ± 9.1	0.001

	Control	3٤ ± 11	
M(%)	Case	13 ± 6	0.002
	Control	7 ± 2.3	
E(%)	Case	5 ± 3.1	0.000
	Control	2 ± 1	
B(%)	Case	0.4 ± 0.6	0.001
	Control	0 ± 0.3	

Figure (3.1): Hematological parameters among benzene workers as case group and control

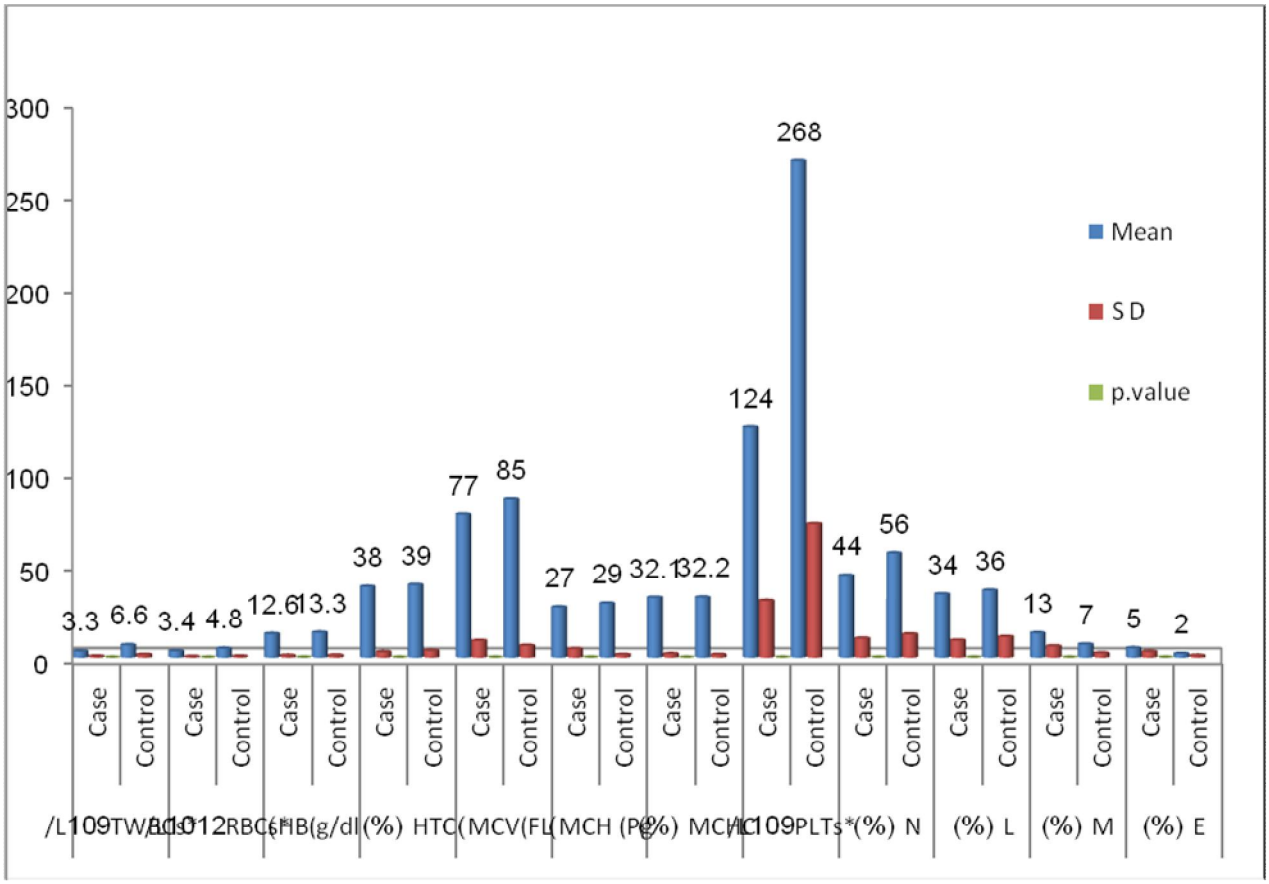

Table (3.4): The relation between hematological parameters of workers in benzene station and duration of work.

Parameters	1-5 years	6-10 years	11-15	P. value
	mean ± SD	mean ± SD	mean ± SD	
TWBCsx10^9/L	3.5 ± 0.6	3.5 ± 0.4	2.8 ± 0.4	0.00
RBCsx10^{12}/L	3.5 ± 0.3	3.4 ± 0.3	2.9 ± 0.5	0.00
HB(g/dl)	13.2 ± 0.3	12.2 ± 0.6	11.3 ± 0.6	0.00
HCT(%)	39.8 ± 1.1	36.7 ± 1.9	34.1 ± 1.9	0.00
MCV(FL)	82.5 ± 3.5	73.0 ± 4.6	64.1 ± 4.0	0.00
MCH(Pg)	30.5 ± 9.2	23.7 ± 2.0	20.0 ± 2.0	0.00
MCHC(%)	30.5 ± 1.0	32.0 ± 1.2	33.5 ± 1.5	0.00
PLTs x10^9/L	135.0 ± 27.0	127.0 ± 20.0	90.0 ± 18.0	0.00
N(%)	43.0 ± 10.0	45.0 ± 10.0	42.0 ± 9.0	0.00
L(%)	39.0 ± 8.2	33.0 ± 7.0	33.0 ± 10.0	0.00
M(%)	10.0 ± 6.0	13.0 ± 4.0	18.0 ± 5.0	0.02
E(%)	4.0 ± 3.3	6.0 ± 4.2	7.0 ± 2.2	0.00
B(%)	0.2 ± 0.2	0.3 ± 0.6	0.5 ± 0.7	0.00

Chapter Four

4.1 Discussion.

This study has been carried out in 100 petroleum benzene station workers their age range from (18 – 52) years old and the duration in work ranges from 1 – 15 years, this people work for three days / week for 12 hours / day. The two hundred blood samples were collected (100 sample from workers in the benzene station and 100 samples from control). In Khartoum state in the period from April to June 2014.

This study aimed to assess if there is any change in hematological parameters due to inhalation of benzene products.

The result of the study shows all hematological parameters (total white blood cells red blood cells and platelets) were decrease among workers exposed to benzene compared to control, with some exception of mean of monocyte, eosinophil and basophil which higher among exposed worker in benzene station, that mean benzene may leas to panctopenia, Eosnophilia, Basophilia and monocytosis, also the result show there is association between period of exposure to benzene and pancytopenia, Eosinophilia, Basophilia and monocytosis which increase by increasing of working duration.

- In the study done in turkey by M. Dincol, K. Akgun, T. Erdemsond and Dincol (1971), the result of this study the effect of benzene can causes pancytopenia, eosinophilia and Basophilia and results of this study which agreed with m results.

- In the another study done in Negeria by A. M. OKORD; et al (2006), this study found the inhalation of petroleum fumes causes significant decrease in white blood cells count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration there hemoglobin and mean corpuscular volume when compared with control, and most subjects exposed for longer than two years had significantly lower value of red blood cell count, hemoglobin concentration and hematocrit than those exposed for less than two years and this result which was agreed with my result.

4.2 Conclusion:

This study conducted:

1- There is significant effects from inhalation of benzene product on RBCs, count and their indices (HCT, HGB, MCV, MCH, MCHC) show reduction and this effects increase by increasing of duration.

2- There is significant decrease on WBCs count by inhalation of benzene products with Eosinophilia, Basophilia and Monocytosis and this effects increase by increasing of working duration.

3- There is significant effect on platelets count show as thrombocytopenia and this effect increase by increase of work duration.

4.3 Recommendations:

This study recommended.

- Using protective cloths to workers in benzene stations.
- Store benzene outside your home, this also reduce fire hazard and amount of vapor entering your home to prevent inhalation.
- Determine the limit period for workers.
- Do regular monitor and follow up for workers.
- Do medical examination for workers regularly and save the result.
- Use protective tools for workers in benzene station.

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Appendix - 1

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Questionnaire

Name . ……..

Age . ……..

Duration of work at this station. …….

Any disease ?

Yes …….. no …….

Worker sign . …….

Appendix-2

Sysmex device