1. Introduction and literature review

1.1 Introduction

1.1.1 Defintion of blood

Blood is described as a specialized connective tissue,which circulates in a closed system of blood vessels. (Monica.C, 2009)

1.1.2 Blood components

Plasma is 55% of the total blood ,plasma cosist of albumin,globulin,water,electrolyte and many other organic and inorganic substances. (Monica.C, 2009)

Blood cells is 45% of total blood and encompass;White blood cells(WBCs),Red blood cells(RBCs),and Platelets(Plts). (Monica.C, 2009)

1.1.3 Function of blood

-Respiration: transport of oxygen from the lung to tissues and carbon dioxide from tissues to the lungs.

-Excreation:transport of metabolic waste to the lungs,kidneys,skin and intestine for removal.

- Maintain of normal acid –base balance.

-Nutrition of body .

-Part of immune system. (Monica.C, 2009)

1.1.4 Haemopoiesis

Is the general aspect of blood cells formation. (Monica.C, 2009)
Haemopoiesis occurs at different anatomical sites the course of development from embryonic life to adult life this site is,

up to 2 month of gestation.The haemopoiesis occurs in yolk sac of the embryo.This period called (Myeloblastic period). (Monica.C, 2009)
2-7 month of gestation, this period called (Haepatic period). (Monica.C, 2009)

Only important site of all hemopoiesis site after birth,an exception is lymphocyte production which occur in other organ in addition to the bone marrow.This period called(Myeloid period). (Monica.C, 2009)

1.1.5 Development of haemopoiesis

The general most commonly accepted view is that blood cells development from small population of stem cells. (Dacie and Lewis, 2006)
The General characteristic of stem cells:-

1.Pluripotentential cell.

2.They can maintain their number by self replication.

3.They give rise to precursor of one or more various blood cell serious.
4.The immune system cells are derived from stem cells.

5. Stem cell phenotypes are unknown.

6.The size of stem cells is similar to the size of small lymphocytes.

7.Some immunological tests show:CD34+,CD38-.(Dacie and Lewis, 2006)

1.1.6 Stages of haemopoiesis

1.1.6.1 Erythropoiesis

Red cells are produced by proliferation and differential of precursor which known as erythroblasts.Normoblasts are referred to erythroblasts when their morphological features are with in normal limit. (Dacie and Lewis, 2006)

In the proliferation stage capacity is lost and haemoglobin becomes predominant protein in cytoplasm. (Dacie and Lewis, 2006)

In differentiation stage,the size of erythroblasts decreases progressively and character of nucleus and cytoplasm changes as the cells proceed to word the point. (Dacie and Lewis, 2006)

1.1.6.1.1 Proerythroblast

There are several nucleuses in the nucleus,it occupies most of the cell and round in shape the chromatin in the nucleus consist of network of fine red purples strands and size about 14-20Mm. (Dacie and Lewis,(2006)

Peripheral cytoplasm is more basophililc than myeloblast.Proerythroblast undergo is rapid division and give rise to basophililc erythroblast. (Dacie and Lewis, 2006)

1.1.6.1.2 Basophilic erythroblast

It also called early erythroblast,rounded in shape,(12-16Mm) in diameter,more basophilic than proerythroblast,it is occupied relative large proportion of cells;it is differ from nucleus of promyeloblast by having course and more basophilic chromatin strands. (Dacie and Lewis, 2006)

1.1.6.1.3 Polychromatic "intermediate"erythroblast

Round in shape and has(12-16)Mm in diameter,

it derived from the mixture of the basophilic (RNA)and acidophilic (Hb),nuclear chromatin is coarser and deeply basophilic clump,the proliferation activity ceases after this stage. (Dacie and Lewis, 2006)

It is called intermediate erythroblast because it occupied a position in maturation pathway between early immature which characterized by absent of proliferation and predominant "acidophilic Hb".(Dacie and Lewis, 2006)

1.1.6.1.4 Orthochromatic erythroblast

It is final stage of maturation of the nucleated red cell, have diameter between (8-12)Mm, nucleus is relatively small and have homogeneous blue-black appearance,nucleus extended from orthochromatic to form the reticulocyte acidophilic cytoplasm due to active synthesis of haemoglobin,it contains mitochondria and ribosome. (Dacie and Lewis, 2006)

1.1.6.1.5 Reticulocyte

Reticulocyte has the same biconcave discoid shape as mature red cells.They different from mature cells by slightly grater volume and diameter than the mature cells. The Cytoplasm of reticulocyte is similar in staining to the orthochromatic erythroblasts which are distinguished from mature red blood cells by diffuse basophilic hemoglobin. (Dacie and Lewis, 2006)

1.1.6.1.6 Mature red blood cells

Similar to reticulocyte but have no basophilic granules(RNA) round or biconcave in shape , the cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the

red color of the cells. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network. (Dacie and Lewis, 2006)

1.1.6.2 Granulopoiesis

White blood cells production. (Dacie and Lewis, 2006)

1.1.6.2.1 The Myeloblast

large size (15-20Mm) in diameter,round to oval shape, nucleus occupies a large proportion of the cell ,nuclear chromatin is arranged in a five net work of red purple stand with occasional small aggregates;nuclei are typically prominent while two or three is the usual number,there may be up to six nuclei,Cytoplasm is moderately basophilic and there is no granules. (Dacie and Lewis, 2006)

1.1.6.2.2 The Promyelocyte

The feature of this cell are similar to that myeloblast except for the development of some cytoplasmic granules and slightly more coarse appearance of the chromatin. (Dacie J and Lewis .S.M, 2006)

1.1.6.2.3 The Myelocyte

Nucleus /cytoplasm ratio is greater than the promyelocyte,nuclei are not larger present,chromatin more aggregated than in promyelocyte,cytoplasm is less basophilic and has prominent cytoplasmic granules. (Dacie and Lewis, 2006)

1.1.6.2.4 The Metamyelocyte

In this stage the nucleus becomes indented and assumes a kidney shape and granules are prominent in the cytoplasm. (Dacie and Lewis, 2006)

1.1.6.2.5 The Band form (Stab)

The degree of indentation of nucleus of it is grater than 50% of nuclear diameter. The cytoplasmic granules are identical to those in mature segmented form. (Dacie and Lewis, 2006)

1.1.6.3 Lymphopoiesis

The lymphocytes pass through series of developmental changes in course of evolving into various lymphocytes sub-population. (Dacie and Lewis, 2006)

1.1.6.3.1 The lymphoblast

The ratio of diameter of the nucleus to that of the cell tends to be greater than myeloblast, number of nuclei in nucleus is fewer than myeloblast. Lymphoblast is actively dividing cell. (Dacie and Lewis, 2006)

1.1.6.3.2 The large lymphocyte

Have (12-16Mm) in dimeter,has round outline shape,round or slightly indented nucleus,chromatin is more clumped than in myeloblast,cytoplasm is pale blue and more abundant than in the lymphoblast. (Dacie and Lewis, 2006)

1.1.6.3.3 The Small lymphocyte

Have (9-12Mm) in diameter,nucleus is rim uncalculating around or marginally indented nucleus which contains deeply staining,heavily clumped chromatin, cytoplasm is thin,deeply basophilic. (Dacie and Lewis, 2006)

1.1.6.4 Thrombopoiesis

Platelets are formed in bone marrow by megakaryocyte,and are subsequently released in vascular component and play essential role in homeostasis. (Dacie and Lewis, 2006)

1.1.6.4.1 Megakaryoblast

Is a precursor cell to a promegakaryocyte, which in turn becomes a megakaryocyte during haematopoiesis. It is the beginning of the thrombocytic series. (Dacie and Lewis, 2006)

1.1.6.4.2 Promegakaryocyte

Larger than precursor cells becomes it has undergone endo reduplication.Endo replication is nuclear replication without division of the cells and is a characteristic feature of the more mature membrances of megakaryocytic series. (Dacie and Lewis, 2006)

The nucleus may be lobulated and the chromatin is more deeply basophilic than in the megakaryoblast, cytoplasm deeply basophilic containing some basophilic granules. (Dacie and Lewis, 2006)

1.1.6.4.3 Megakaryocyte

Has (30-90Mm)in dimeter,coarsely clumped chromatin,cytoplasm is larger expanse,stain light blue,contain many small red purple granules. (Dacie and Lewis, 2006)

1.1.6.4.4 The platelets

Small and are discoid in shape, (1-4 Mm) in dimeter,cytoplasm is light blue and contains small red purple granules which are centrally located in platelets in the blood film. (Dacie and Lewis, 2006)

1.1.7 Haemoglobin

It is conjucated protein has molecular weight 64.000 dalton consist of two pairs of globulin chains (Haem + globulin) each pairs is attached to haem molecule located in RBCs function as oxygen carries from lung to tissue and return back carbon dioxide from tissue to lung. (Dacie and Lewis, 2006)

1.1.8 Anaemia

It is reduction of the RBCs count, Hb concentration lower than lower extereme of normal range according to sex and age. (Dacie and Lewis, 2006)

1.1.8.1 Classification of anaemia

The several kinds of anemia are produced by a variety of underlying causes. It can be classified in a variety of ways, based on the morphology of RBCs, underlying etiologic mechanisms, and discernible clinical spectra, to mention a few. The three main classes include excessive blood loss (acutely such as a hemorrhage or chronically through low-volume loss), excessive blood cell destruction (hemolysis) or deficient red blood cell production (ineffective hematopoiesis). (Dacie and Lewis, 2006)

1.1.9 Leucopenia

Leukopenia (also known as leukocytopenia, or leucopenia, is a decrease in the number of white blood cells (leukocytes) found in the blood, which places individuals at increased risk of infection. (Dacie and Lewis, 2006)

1.1.10 Thrombocytopenia

The terms thrombocytopenia and thrombopenia, refer to a relative decrease of platelets in blood. (Dacie and Lewis, 2006)

A normal human platelet count ranges from 150,000 to 450,000 platelets per microlitre of blood. These limits are determined by the 2.5th lower and upper percentile, so values outside this range do not necessarily indicate disease. One common definition of thrombocytopenia is a platelet count below 50,000 per microlitre. (Dacie and Lewis, 2006)

1.1.11 Pancytopenia

Pancytopenia is a medical condition in which there is a reduction in the number of red and white blood cells, as well as platelets. (Dacie and Lewis, 2006)

If only two parameters from the full blood count are low, the term bicytopenia can be used. The diagnostic approach is the same as for pancytopenia. (Dacie and Lewis, 2006)

1.1.12 Complete Blood Count(CBC)

Complete blood count is a very common test that uses to evaluate the three major type of cell in blood ,red blood cells,white blood cells,and platelets. (Dacie and Lewis, 2006)

Aim of This test :

1.Screening test to check some blood disorders"Anaemia,infection,and inflammation.

2.Use to determine the general health status of people.

3.Use to monitoring and flowing up the treatment and drugs effects. (Dacie and Lewis, 2006)

The related tests**:-**

- Haemoglobin estimation(Hb): is the amount of hemoglobin in the blood, expressed in grams per decilitre. (Dacie and Lewis, 2006)
- Haematocrit(HCT):haematocrit or packed cell volume (PCV) , this is the fraction of whole blood volume that consists of red blood cells. (Dacie and Lewis, 2006)
- Red blood cell count(RBCs):total red blood cells is the number of red cells is given as an absolute number per litre. (Dacie and Lewis, 2006)
- Red blood cell indicies:-

- MCV: Mean corpuscular volume (MCV) , the average volume of the red cells, measured in femtolitres. (Dacie and Lewis, 2006)
- MCH: Mean corpuscular hemoglobin (MCH) , the average amount of hemoglobin per red blood cell, in picograms. (Dacie and Lewis, 2006)
- MCHC: Mean corpuscular hemoglobin concentration (MCHC) ,the average concentration of hemoglobin in the cells. (Dacie and Lewis, 2006)
    - Total white blood cell count(TWBCs): Total white blood cells , the white cell types are given as a percentage and as an absolute number per litre. (Dacie and Lewis, 2006)
    - White blood cell differential count: is comprised of several different types that are differentiated, or distinguished, based on their size and shape. The cells in a differential count are

granulocytes, lymphocytes, monocytes, eosinophils, and basophils. (Moroni *et al*, 2011)

- Platelets: Platelet numbers are given, as well as information about their size and the range of sizes in the blood. (Dacie and Lewis, 2006)
- Examination and evaluation of a peripheral blood picture: Use to detect morphology and size of blood cell.(Dacie and lewis, 2006)

1.1.13 Radiation

Radiation is energy in the form of waves or streams of particles.There are of many kinds of radiation all around us.When people hear the word radiation,they often think of atomic energy, nuclear power and radioactivity,but radiation has many other forms.Sound and visible light are familiar forms of radiation, other types include ultraviolet radiation (that produce a suntan),infrared radiation (a form of heat energy),and radio and television signals. (Williams *et al*, 2010).

1.1.13.1 Ionizing radiation

It is high-energy radiation capable of producing ionization in substances through which it passes.It include non-particulate radiation,suh as x-rays and radiation produced by energetic charged particles,such as alpha and beta rays and neutrons as form a nuclear reaction. (Williams *et al*, 2010).

1.1.13.2 Alpha radiation

Alpha radiation consists of alpha particles that are made up of two protons and two neutrons each and that carry a double positive charge.Due to their

relatively large mass and charge,they have an extremely limited ability to penetrate matter. (Williams *et al*, 2010).

1.1.13.3 Beta radiation

Beta radiation consists of charged particles that are ejected from an atoms nucleus and that are physically identical to electrons.Beta particles generally have a negative charge,are very small and can penetrate more depply than alpha particles. (Williams *et al*, 2010).

1.1.13.4 Neutron radiation

Apart from cosmic radiation,spontaneous fission is the only natural source of neutron (n).Neutrons are able to penetrate,tissues and organs of the human body when the radiation source is outside the body.Neutrons can also be hazardous if neutron-emitting nuclear substances are deposited inside the body. (Williams *et al*, 2010).

1.1.13.5 Photon radiation

Photon radiation is electromagnetic radiation.There are two types of photon radiation of interest for the purpose of this document: gamma and X-ray. (Williams *et al*, 2010).

X-rays are also ionizing radiation, penetrate the skin ,virtually identical to gamma rays, but not nuclear in origin. However the effect of this radiation does not depend on its origin but on its energy. (Williams *et al*, 2010)

1.1.13.6 Uses of radiation In medicine

Radiation and radioactive substances are used for diagnosis, treatment, and research. X-rays, for example, pass through muscles and other soft tissue but are stopped by dense materials. This property of X-rays enables doctors to

find broken bones and to locate cancers that might be growing in the body . (Moroni *et al*, 2011).

Other forms of radiation such as radio waves, microwaves, and light waves are called non-ionizing. They don't have as much energy and are not able to ionize cells. (Moroni *et al*, 2011).

1.1.13.7 Exposure To Radiation

Radiation exposure may be internal or external, and can be acquired through various exposure pathways. (Rust *et al* , 1954)

Internal exposure to ionizing radiation occurs when a radionuclide is inhaled, ingested or otherwise enters into the bloodstream (e.g. injection, wounds). (Rust *et al* , 1954).

External contamination may occur when airborne radioactive material (dust, liquid, aerosols) is deposited on skin or clothes. This type of radioactive material can often be removed from the body by simply washing. Exposure to ionizing radiation can also result from external irradiation (e.g. medical radiation exposure to X-rays). (Rust *et al* ,1954).

1.1.13.8 Effects of ionizing radiation on human body

The radiation affects human body in highly complicated processes. Various degrees of 4biological effects, from damage to death of living tissues, involve a number of pathological changes in human cells.When exposed to ionizing radiation, large molecules such as nucleic acid and proteins in the cells will be ionized or excited. This may cause changes in the molecular structures which then affect the function and metabolism of the cells. (Dacie and Lewis , 2006).

Diagnostic X-rays (primarily from CT scan due to the large dose used)

increased the risk of developmental problem and cancer in those exposed. (Dacie and Lewis, 2006).

1.1.14 Benzene

1.1.14.1 Definition

A colorless volatile liquid hydrocarbon present in coal tar and petroleum, used in chemical synthesis. Its use as a solvent has been reduced because of its carcinogenic properties. (Langley A, 2005).

1.1.14.2 Physical properities

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

1.1.14.3 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 1 hydrogen atom attached to each carbon atom. Because its molecules contain only carbon and hydrogen atoms, benzene is classed as ahydrocarbon. (Langley A, 2005).

Benzene is a colorless and highlyflammable 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 A, 2005).

1.1.14.4 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 A, 2005).

1.1.14.5 Distribution of benzene

After entry into the human organism, benzene is distributed throughout the body and, owing to its lipophilic nature, accumulates preferentially in fat-rich tissues, especially fat and bone marrow. In humans, benzene crosses the blood–brain barrier and the placenta and can be found in the brain and umbilical cord blood in quantities greater than or equal to those present in maternal blood. (Langley A, 2005).

1.1.14.6 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 tissues, such as the bone marrow. (Langley A, 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 muconaldehyde. The genotoxic activity of benzene metabolites 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 A, 2005).

1.1.14.7 Benzene exposure in the work place

Exposure to benzene occurs by three main ways:

1.Breathing (inhalation exposure).

2. Eating and/or drinking contaminated food or water.

3.Absorption through the skin (contact with skin). (Katzung and Diuritic, 2004)

1.1.14.8 Hazard effects of exposure to benzene

Health effect are divided according to:

1. Duration time
2. Level of benzene. (Katzung and Diuritic, 2004)

1.1.14.9 Long-term exposure to benzene

Haematological effect (in blood and blood forming organs),prolonged exposure to benzene can cause a serious condition where the number of circulating erythrocytes , leucocytes and reduced (pancytopenia) at this stage effects are thought to be readily reversible.However continued exposure can result in a plastic anaemia or leukaemia. (Monica.C, 2009)

1.1.14.10 Benzene related to leukaemia

The International Agency for Research on Cancer (IARC) has concluded that there is sufficient evidence for carcinogenicity of benzene to humans.(Stuene *et al* , 1996)

Benzene able to cause acute myeloid leukaemia (AML),myelodysplastic syndrome(MDS),chronic lymphoid leukaemia(CLL), aplastic anaemia (A.A) but more frequently it cause (AML),(MDS) (Stuene *et al* , 1996).

1.2 Literature Review

In 2002 the department of physiology collage of medicine,King Saud University ,Study the effect of X-ray Technition ,stated that the mean value of platelet count was significantly decreased in X-ray Technition when compared to controls.however no significant difference was observed in RBC and WBC count between the groups. (Meo S.A,2002)

In 2008 Jondishapour University of Medical Science (AJUMS),Ahwaz,Iran study the haematological profile change in radiation filed workers stated that platelet and white blood cell count were decreased in radiation filed workers with an increased duration of exposure.Radiation filed a statistically significant decrease in platelets and white blood cell counts,respectively when compared to controls. However no significant difference was observed in the rest of haematological parameters between the groups. (Fakher *et al* ,2008)

In a follow up study done in turkey on 44 pancytopenic patient due to chronic exposure to benzene indicate that most patients died from complication of a plastic anaemia or from leukaemia and there is relationship between age,duration of exposure and pancytopenia out come. (Wallace *et al*,1987)

In "1999" many researchers in the "USA" reported that exposure to benzene may lead to blood changes including a steep decline in disease.Fighting white blood cells have been found in workers persistently exposed to low levels of benzene,a common industrial chemical known to pose leukaemia at high concentrations.In the sciences study the

researchers analyzed blood sample from 240 workers who were routinely exposed to benzene laced glue in Chines shoe factory,they reported significant reduction in the total white blood cells count that other people who un exposure to benzene. (Orshoren *et al*,2001)

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 shows features of preleukaemia phase in their blood. (Baker *et al*,1981)

In a study done on "50-100" mice revel that treatment with benzene prolongs the survival time of mice with chloro leukemia this caused by destruction of many of the leukaemic cells,diminution in the size of spleens infiltrated,diminution of subcutaneous tumors composed of leukemic cells. This indicate that chloroleukemia cells are sensitive to the treatment with benzene but the recurrence of most subcutaneous tumors and ultimate death of animals from generalized chloroleukemia indicate temporary effect of benzene.(Martin,1998)

No dose-response effect was observed for most of the examined hematological outcomes (WBC, lymphocytes, neutrophils, monocytes, RBC, Hb, HCT, MCV, platelets and MPV). The eosinophil count was inversely related to benzene exposure only among smokers. Conversely, basophils increased with increasing exposure. No effect on benzene hematotoxicity was found for any of the investigated polymorphisms.(55%Pesatori A, 2009)

1.3 Rational

The radiation and benzene affects human body in highly complicated processes.Various degrees of biological effects, from damage to death of living tissues, involve a number of pathological changes in human cells.When exposed to ionizing radiation or benzene ,large molecules such as nucleic acid and proteins in the cells will be ionized or excited. This may cause changes in the molecular structures which then affect the function and metabolism of the cell.

This study will provide information about change in haematological cells in Sudanes people who exposed to radiation and benzene in Khartoum state to detect the effects of exposure to radiation waves in radiation and benzene toxicity workers compared with control, hoping to provide a reference data about them and providing health programs to inform radiation and benzene workers about the health problems associated with radiation wave and benzene toxicity.

1.4 Objective

1.4.1 General objective

Measured some haematological parameters of among radiation and benzene station workers in Khartoum state and their relation to age and duration of the experience in workers

1.4.2 Specific objective

1.Measured RBCs Count, Hb , RBCs indicis(MCV, MCH, MCHC).

2. Measured TWBCs count, Differential leukocyte count).

3.Count PLTs number.

2. Materials and Method

2.1 Study design

This is an observational, description ,cross-sectional study conducted in radiation and benzene station in Khartoum state during the period from March to May, 2014 .

2.2 Study population

- Radiation workers.

Workers of petroleum stations whom are usually males.-

2.3 Inclusion and Exclusion criteria

-Sudanes Radiation worker.

-Petrolum worker.

-Appearently healthy individual.

2.4 Data Collection

Sample were collected and investigated to determine the CBC value.A questionnaire was used to obtain gender,age,address,time starting job,the years which has worked,rate of working/week,rate of working hours/day,other disease.

2.5 Data analysis

The data was computed and analyzed to obtain the mean,standard deviation and frequencies using statistical package for social science computer program (SPSS) computer program.

3.6 Ethical consideration

It was considered that all information obtained 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 arised especially when the collection technigue was applied.

3.7 Sample collection

Single and non traumatic veni puncture.

It is non probability sampling technique known as convenience sample collected according to certain criteria.

2.8 Materials

-Ethylene diamine tetra acetic acid (EDTA) containers.

- Cotton.

-Alcohol(70%).

-Syringes and tourniquet.

2.9 Principle of sysmex

The coulter principle is based on the following:

Particles suspended in an isotonic diluents when drawn through an aperture which has an electric current flowing through it will cause a measurable drop in voltage which is proportional to the size of the particle passing through the aperture is constant the particle can be quantified per unit volume.This also called electrical impedence.(Monica.C, 2009)

2.10 Methods of sysmex

2.10. 1 Whole blood mode

40ul of blood measured by the sample rotor value is diluted into 1:500 with 1.996ul of diluents and brought to the mixing chamber as diluted sample (1^{st} step dilution).(Monica.C, 2009)

Out of the 1:500 dilution sample 40ul is measured by the sample rotor value,diluted into 1:25000 with 1.960ul of diluents then transferred to the RBCs/plt transducer chamber is aspirated through the aperture.At this time RBCs and plt are counted by the DC detection method.At the same time,haematocrit (HCT) value is calculated by RBCs pulse height detection method.(Monica.C, 2009)

2.11 Method of preparation and staining of blood films

2.11.1 Requirements

1.Slides (76 ×26 mm and approximately 1.2 mm thick) (CITOTEST LABWARE MFG.CO.LTD.CHINA).

2.Spreader (from a glass slide that has a smooth end).

3.Romanowsky stains (RAL stain).

2.11.2 Procedure

Blood film was prepared from fresh blood with no anticoagulant added or from ethylene diamine tetra acetic acid (EDTA) anticoagulanted blood.(Dacie and Lewis, 2006)

A small drop of blood was placed in the center line of slide about 1 cm from one end then without delay a spreader was placed in front of the drop at an angle of about 30 degree to the slide and move it back to make contact with the drop, the drop was spread out quickly along the line of contact.(Dacie and Lewis, 2006)

With a steady movement of the hand, the drop of the blood was sprit along the slide,after that the films was allowed to dry in the air,then the film was labled immediately after spreading.(Dacie and Lewis, 2006)

2.11.3 Staining of the blood film

2.11.3.1 Principle of RAL stain

RAL555 Kit is fast-acting variation of may-grun wald Giemsa stain.In an aqueous buffered medium,this kid enables differential staining of blood smear ,morphology study of leukocyte,study of parasites.

2.11.3.2 Kits components

1.Bottle(1):Fixative-RAL555 X 100ml.

2.Bottle(2):Eosin-RAL555 X 100ml.

3.Bottle(3):Blue-RAL555 X 100ml.

2.11.3.3 Staining procedure

Use "75×25 mm and 1mm thick" clean slides and forested at one end to facilities labeling were used.

Asmall drop of blood was placed in the center line of slide about 1cm from one end.

Without delay a spreader was placed in front of drop at an angle of about 30 degrees to slide and move it back to make contact with the drop so the drop will spread out quickly a long the line of contact.

With the steady movement of the hand,spread the drop of blood was placed a long slide (the spreader must not be lifted until the last trace of blood and was dry by air.

The slide was dipped 5x1 second in solution (1) surplus solution was drained on to filter paper.

The slide was dipped 5x1 second in solution (2) surplus solution was drained on filter paper.

The slide was dipped 5x1 second in solution (3) rinse the slide with distilled water.

Under tab water the slide was washed and the back of the slide was cleaned then air dried.

The slide was been examined under microscope x100 for comment on the cell morphology. (Dacie and Lewis, 2006)

3.Result

Table (3.1): Study population according to age and sex

Destribuation of group under study by age and knowledge of the number of participants of males and females in each field workers

	N	Age (Mean)	Minimum (years)	Maximum (years)	Male (%)	Female (%)
Radiation	50	31	22	52	40	60
Benzene	50	29	18	52	100	-
Control	50	30	17	52	50	50

Table (3.2): Frequence of Age group in studied patients group (Radiation and Benzene)

Segmentation under study by age

Age group (years)	Frequence
Less than 20	7
21-30	55
30-40	23
More than 40	15
Total	100

Table (3.3): Frequence of Working duration in studied patients group (Radiation and Benzene)

Segmentation under study by the number of working hours

Working Duration (yea	Frequence
Less than 5	52
5-10	25
More than 10	23
Total	100

Table (3.4): Comparison of CBC parameters between working in Radiation, Benzene and Control group

There was a significance decrease in value of Platelets to radiation workers compared to control.

There was a significance decrease in value of all parameter of benzene workers compared to control while significant increased Esinophils and basophils.

Parameters	Population	Mean	Std.Deviation	P value
TWBCs (× 10³) cumm	Radiation	5.8	1.8	0.4
	Control	5.5	1.4	-
	Benzene	3.4	0.6	0.0
RBCs (×10³) cumm	Radiation	4.7	0.4	0.3
	Control	4.8	0.4	-
	Benzene	3.4	0.4	0.0
Hb(g/dl)	Radiation	13.2	1.4	0.8
	Control	13.3	1.1	-
	Benzene	12.6	0.9	0.0
HCT(%)	Radiation	40.8	3.9	0.6
	Control	40.4	3.5	-
	Benzene	38.0	2.8	0.0
MCV(Fl)	Radiation	86.4	4.9	0.3
	Control	87.3	4.9	-
	Benzene	77.3	8.8	0.0
MCH(Pg)	Radiation	28.4	1.9	0.3
	Control	29.5	7.3	-
	Benzene	27.2	9.7	0.0
MCHC(%)	Radiation	32.1	1.9	0.6
	Control	32.3	1.4	-
	Benzene	32.9	1.6	0.0
PLTs (×10³) cumm	Radiation	173	59.3	0.0
	Control	283	60.7	-
	Benzene	125	32.1	0.0
N(%)	Radiation	50	12.9	0.1
	Control	54	11.3	-
	Benzene	43	10.1	0.0

L(%)	Radiation	38	11.7	0.6
	Control	33	8.0	-
	Benzene	37	9.3	0.0
M(%)	Radiation	7	4.1	0.5
	Control	7	1.7	-
	Benzene	13	6.1	0.0
E(%)	Radiation	3	4.1	0.8
	Control	2	1.0	-
	Benzene	5	3.5	0.17
B(%)	Radiation	1	1.1	0.2
	Control	1	0.2	-
	Benzene	.3	0.6	0.87

P value and statistical significance:

The two-tailed P value is less than 0.05

By convential criteria ;this difference is considered to be extremely statistically significant.

Table (3.5): Comparison CBC parameters between Radiation and Benzene

There was a significance variation (decrease in value of all parameter) of benzene workers compared to radiation workers.

Parameters	Population	Mean	Std.Deviation	P value
TWBCs (10^3) cumm	Radiation	5.8	1.8	.000
	Benzene	3.4	0.6	
RBCs (× 10^3) cumm	Radiation	4.7	0.4	.000
	Benzene	3.4	0.4	
Hb(g/dl)	Radiation	13.2	1.4	.000
	Benzene	12.6	0.9	
HCT(%)	Radiation	40.8	3.9	.000
	Benzene	38.0	2.8	
MCV(FL)	Radiation	86.4	4.9	.000
	Benzene	77.3	8.8	
MCH(Pg)	Radiation	28.4	1.9	.000
	Benzene	27.2	9.7	

MCHC(%)	Radiation	32.1	1.9	.000
	Benzene	32.9	1.6	
PLTs (× 10³) cumm	Radiation	173	59.3	.000
	Benzene	125	32.1	
N(%)	Radiation	50	12.9	.000
	Benzene	43	10.1	
L(%)	Radiation	38	11.7	.000
	Benzene	37	9.3	
M(%)	Radiation	7	4.1	.000
	Benzene	13	6.1	
E(%)	Radiation	3	4.1	.000
	Benzene	5	3.5	
B(%)	Radiation	1	1.1	.000
	Benzene	0.3	0.6	

P value and statistical significance:

The two-tailed P value is less than 0.05

By convential criteria ;this difference is considered to be extremely statistically significant.

Table (3.6): Measure of Association between age group and pathogencity of Radiation and Benzene

There was a significance variation (decrease) in PLTs in all age group of radiation workers.

There was a significance variation (decrease) in all parameter between age group of benzene workers.

Parameters	**Population**	**Less than 20**		**21-30**		**31-40**		**More than 40**		**P value**
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
TWBCs (× 10³) cumm	Radiation	-	-	5.7	1.8	6.4	2.0	5.5	1.2	.44
	Benzene	3.6	0.4	3.5	0.6	3.3	0.5	2.7	0.4	.02
RBCs (× 10³)	Radiation	-	-	4.6	0.4	4.8	0.5	4.6	0.5	.47
	Benzene	3.7	0.3	3.5	0.3	3.3	0.3	2.8	0.4	.00

cumm										
Hb(g/dl)	Radiation	-	-	13.0	1.4	13.5	1.7	13.5	1.0	.58
	Benzene	13.5	0.3	12.9	0.6	12.1	0.8	11.2	0.6	.00
HCT(%)	Radiation	-	-	40.4	3.8	41.5	4.7	40.8	2.9	.58
	Benzene	40.6	1.1	38.9	1.8	36.5	2.5	33.8	2.1	.00
MCV(FL)	Radiation	-	-	86.9	4.4	85.6	6.2	86.0	3.9	.69
	Benzene	87.3	2.4	80.5	5.2	72.0	4.1	62.0	3.5	.00
MCH(Pg)	Radiation	-	-	28.4	1.7	28.0	2.5	29.1	1.5	.45
	Benzene	38.6	2.8	27.4	2.7	23.6	2.2	19.5	1.8	.00
MCHC(%)	Radiation	-	-	32.1	0.9	32.5	1.4	31.3	3.4	.30
	Benzene	32.0	1.4	31.7	1.0	32.6	1.5	34.9	1.5	.00
PLTs	Radiation	-	-	203	63	142	13	122	7	.00
(× 10^3) cumm	Benzene	150	43	131	26	113	18	95	28	.00
N(%)	Radiation	-	-	49	12	49	14	51	14	.95
	Benzene	45	17	43	8	44	8	40	8	.00
L(%)	Radiation	-	-	40	11	37	12	35	11	.54
	Benzene	37	10	39	8	34	8	36	11	.00
M(%)	Radiation	-	-	6.9	3.6	6.8	4.1	8.3	5.9	.66
	Benzene	13	10	11	4	14	5	18	4	.00
E(%)	Radiation	-	-	2.3	3.2	4.3	5.9	3.5	2.8	.32
	Benzene	3.1	1.8	4.8	4.1	5.5	2.9	6.8	2.4	.00
B(%)	Radiation	-	-	0.6	1.2	0.5	1.0	0.6	0.7	.95
	Benzene	0.4	0.5	0.3	0.6	0.3	0.7	0.4	0.7	.00

P value and statistical significance:

The two-tailed P value is less than 0.05

By convential criteria ;this difference is considered to be extremely statistically significant.

Table (3.7): Measure of Association between working duration and Radiation , Benzene effects

There was a significance decrease in PLTs value by increase working duration of radiation workers.

There was a significance decrease in value of all parameter while increased in esinophils and basophils of benzene workers by increase duration of working.

Parameters	Population	Less than 5 years		5-10 years		More than 10 years		P value
		Mean	SD	Mean	SD	Mean	SD	
TWBCs ($\times 10^3$)cumm	Radiation	5.5	1.6	6.9	2.3	5.4	1.0	.47
	Benzene	3.5	0.6	3.5	0.4	2.8	0.4	.00
RBCs ($\times 10^3$) cumm	Radiation	4.5	0.4	4.9	0.5	4.6	0.4	.10
	Benzene	3.5	0.3	3.4	0.3	2.9	0.5	.00
Hb(g/dl)	Radiation	13.0	1.4	13.8	1.4	13.0	1.3	.25
	Benzene	13.2	0.3	12.2	0.6	11.3	0.6	.00
HCT(%)	Radiation	40.5	3.9	42.0	4.3	39.9	3.5	.34
	Benzene	39.8	1.1	36.7	2.0	34.1	2.0	.00
MCV(%)	Radiation	87.6	3.9	85.4	5.5	85.2	5.4	.24
	Benzene	83.5	3.5	73.1	5.0	64.1	4.5	.00
MCH(%)	Radiation	28.7	1.2	28.1	2.5	28.2	2.3	.57
	Benzene	30.8	11.2	23.7	2.2	20.2	2.1	.00
MCHC(%)	Radiation	32.1	0.9	32.7	1.3	31.5	2.7	.20
	Benzene	31.5	1.0	32.6	1.2	34.5	1.6	.00
PLTs ($\times 10^3$) cumm	Radiation	212	66	151	17	128	10	.00
	Benzene	136	30	126	25	93	21	.00
N(%)	Radiation	48	11	52	14	50	13	.62
	Benzene	43	10	45	10	42	9	.00
L(%)	Radiation	42	10	34	13	38	10	.16
	Benzene	39	9.2	34	7.2	34	10	.00
M(%)	Radiation	6.8	3.6	7.5	3.8	7.2	5.4	.90
	Benzene	11	6.2	13	4.4	18	5.1	.01
E(%)	Radiation	2.2	3.4	3.7	5.5	3.9	3.5	.41
	Benzene	4.3	3.5	6.1	4.3	5.7	2.2	.00
B(%)	Radiation	0.7	1.3	0.4	0.7	0.6	0.9	.71
	Benzene	0.3	0.6	0.3	0.6	0.5	0.8	.00

P value and statistical significance:

The two-tailed P value is less than 0.05

By convential criteria ;this difference is considered to be extremely statistically significant.

4. Discussion, Conclusion and Recommendations

4.1 Discussion

The exposed to ionizing radiation and carcinogenic chemical such as benzene considered two of major causes of haematological effect in blood cells or may lead to pancytopenia.

The study was carried out to determined the effect of ionizing radiation on radiation workers and effect of toxicity of benzene in benzene station workers, the age of studied group range from (18-52) years old and duration of work range from 1-30 years ,these people work for 3 dayes/week to benzene equivalent to 18 hours/day and about 8 hours /day to radiation in Khartoum State ,150 blood samples was collected (50 radiation workers, 50 benzene station and 50 control sample), from the period from march to may 2014, the result show exposure to radiation lead to thrombocytopenia, and TWBCs,Hb,HCT,MCV,MCH,MCHC no affect while exposure to benzene lead to anaemia,leucopenia and thrombocytopenia and addition to esinophilia and basophilia.

After conducting the study and analysis of the result show that difference between control and radiation workers; have no any statistically differenence between radiation workers and control in TWBcs,RBCs,Hb,HCT,RBCs indicies and this result agree with survey on radiation exposure in occupational workers the effect on hematological change ,this study examined the hematological caused by radiation exposure in workers in a medical radiation-exposed environment and no changes in the (red blood cells (RBCs), hemoglobin, white blood cells

(WBCs), monocytes, lymphocytes, neutrophils,) were examined in both the occupational workers and controls by (Ryu *et al*, 2013).

significant decreased in platelets value among radiation exposed workers,(P value = .000) and these result agreed with (Meo S.A ,2002) who stated no significant difference was observed in RBCs and WBCs (P value ≤ 0.05) in x-ray and physiotherapy technitions when compared to controls.

Study reveal statistically significant decrease in lymphocyte and basophil addition to decrease in platelets and this result agree with study (Fakher *et al* , 2008) who stated that the platelet and white blood cell counts were decreased in radiation workers. The study suggests that exposure to X-ray radiation causes atypical changes in the lymphocyte morphology. Further studies are needed to study the long term effects of X-ray radiation on hematological parameters and immunophenotyping of atypical lymphocyte population in X-ray technicians.

On the other hand, The results of haematologic parameters of people exposed to benzene reveal significant decreased of TWBCs,RBCs,Hb,HCT,RBCs indicies (MCV,MCH,MCHC) ,with the exception of Esinophil and Basophil were higher among benzene exposed subjects that mean benzene may lead to pancytopenia .

TWBCs,RBCs,Hb,HCT,RBCs indicies, plts : statistically significantly lower than control and these result are supported many of study such as study done in turkey on 44 pancytopenic patient due to chronic exposure to benzene indicate that most patients died from complication of a plastic anaemia and there is relationship between age, duration of exposure and pancytopenia out come by (Wallace *et al*,1987).

Also agree with other study in china revealed that exposure to benzene produce pancytopenia which complicated to leukaemia in late stage by (Orshoren *et al*,2001) and In USA,the American society of hematology observed that chronic exposure to benzene will lead to hematotoxicity disorders and acute myelogenious leukaemia.

Also statistically significantly lower in neutrophil,lymphocyte,monocyte while statistically increased in esinophil and basophil than control and this result agree with early effects of low benzene exposure on blood cell counts in Bulgarian petrochemical workers,No dose-response effect was observed for most of the examined hematological outcomes, the eosinophil basophils count increased with increasing exposure by**(55%**Pesatori A**,** 2009), as the result of this a myeloproliferative effect of benzene is highly unlikely to explain the observed increase in eosinophils and basophils as it would lead to a concordant depression in all haematopietic populations.

The complains of benzene toxicity in sudan were comparatively lesser than those reported in other countries and this variation may be due to two facts; the 1st one is that,the exposure to benzene will not affect the blood tissues and the 2nd fact is that,in other countries people contact with benzene product is more freguent than sudan and obvious from the observation in sudan people use benzene to fuel their cars,but in other countries people use benzene can affect all the major peripheral blood elements.

4.2 Conclusion

-The CBC parameter show that there was significant difference between radiation workers and control on platelets count, thrompocytopenia increased when increased the number of years of work.

- Observed association between exposure to benzene and pancytopenia which increase by working duration.

4.3 Recommendations

- Furthure investigation for example bone marrow to determine haematological and cytogenetical disorders.
- Regular monitors and follows up for the workers and save the result of follow up .
- Create record of radiation and benzene doses to workers.
- Determine the limit period for petrol for workers.
- The sudanes ministry of health may supply the petroleum stations with safety equipments.

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Sudan University of Science and Technology

college of Graduate Studies

Master program

Ouestionnaire

Evaluation of haematological change in Sudanes Radiation and Benzene workers in Khartoum state.

Sample Number( )

Gender: Male ( ) Female( )

Age:……..

Residence:……..

Profession:……..

Workplace:……..

Period of appointment and work:……..

Rate of working / week: ……..

Rate of working / day :……..

Do you follow safety policies :

Yes( ) No( )

If ansewer is yes,Such as :……..

Do you suffer of any disease:

Yes( ) No( )

If the ansewer is yes, mention disease:……..

Do you suffer of any sign or symptoms:……

Last check up:……

- Result

- Full blood count

Twbcs:

RBCs:

Hb:

MCV:

MCH:

MCHC:

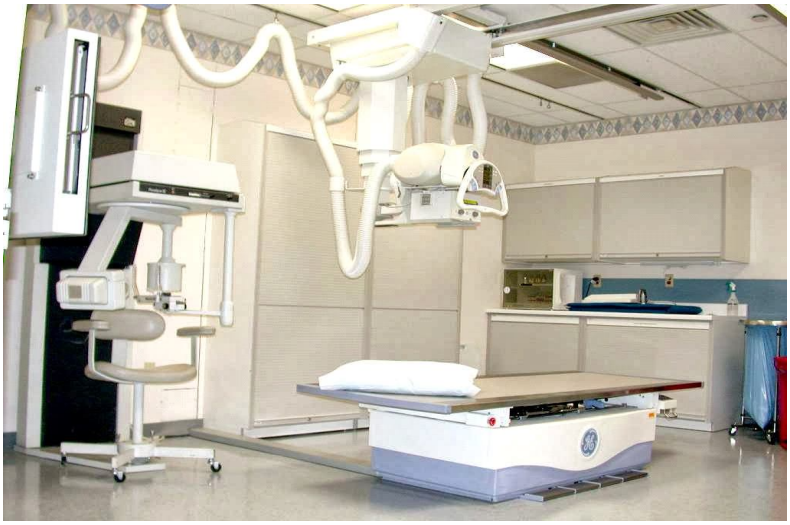
PLT:

N: L: M:

E: B:

- Morphological study

Figure(2.1): Sysmex Instriument



Figure(2.3): X-ray Instrument