بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessment of changes in some hematological parameters among Benzene station workers in Khartoum State

تقييم التغيرات في بعض معايير الدم لدى عمال محطات البنزين بولاية الخرطوم

A dissertation Submitted in Partial Fullfillment for the Requirement of M.s.c Degree in Medical Laboratory Sciences

(Hematology and Immunohematology)

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December 2015

الآيـة

قال تعالى:

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)

صدق الله العظيم

سورة العلق (1-5)

Dedication

To my parents.

To my Sisters

To my Friends

with Love

Acknowledgement

After thanking Allah, who made all things possible and granted me power to finish this work, I would like to express my deep appreciation to my supervisor Dr. Randa Amin Basheer who dedicate a lot of her precious time to give me valuable assistance and guidance throughout this study.

I deeply appreciate my beloved father and mother thank you for always believing in me and loving me unconditionally without your support and love I would not been where I am today.

I owe my heart left wishes to my lovely sisters, brothers, and friends for their encouragement.

Lastly we are obliged to all participants who agreed to participate in this study.

Abstract

Benzene exposure is one of the main health concerns for high risk occupation hazards, it can cause abnormal alterations in the functioning of many vital organs including hematopoietic system.

This is an analytical descriptive cross sectional study intended to find out the possible changes in some hematological parameters in benzene station workers in Khartoum State from August to December 2015.

Forty benzene station workers and 30 control subjects matched for age were enrolled in the study. 2.5 ml of venous blood were collected in K2 EDTA anticoagulant containers. Complete blood count was performed by an automated blood analyzer (Sysmex kx 21N). Data were analyzed by independent t-test and one way ANOVA using SPSS computer program version 16.

The benzene station workers showed significantly higher values than the control; RBCs $\times 10^6/\mu l$(5.02±0.65 vs 4.7±0.3), mean hemoglobin level g/dl(14.5±1.01 vs 13.6±0.54), MCV fl(84.5±8.2 vs 88.9±5.6), and MCHC g/dl(43.5±5.5 vs 32.4±1.4), while HCT %, MCH pg did not vary between the two groups P.value (>0.05).

Platelets $\times 10^3/\mu l$ of benzene station workers showed significantly lower than that of the control (211±77.89 vs 264±86.72).

The Benzene station workers also had increased MXD $\times 10^3/\mu l$ (0.81±0.45 vs 0.6±0.25); in contrast WBCs $\times 10^3/\mu l$, lymphocytes $\times 10^3/\mu l$, and neutrophils $\times 10^3/\mu l$ did not vary between the benzene station workers and the control.

The platelets $\times 10^3/\mu l$ count was gradually reduced as the duration of the work progresses P.value (0.000) as well as lymphocytes $\times 10^3/\mu l$ P.value (0.017), while WBCs $\times 10^3/\mu l$, RBCs $\times 10^5/\mu l$, HBG g/dl, HCT %, MCV fl, MCH pg, MCHC g/dl, MXD $\times 10^3/\mu l$, and Neutrophils $\times 10^3/\mu l$ values did not vary regard to the years of exposures to benzene fumes P.value (>0.05).

The results concluded that there are changes in some hematological parameters among benzene station workers, and suggest that these workers are susceptible to serious hematological changes as the duration of the work progresses.

ملخص الاطروحة

التعرض للبنزين يعد أحد الشواغل الصحية الرئيسية للمهن عالية المخاطر مثل العمل بمحطات البنزين والذي يمكن أن يسبب تغيرات غير طبيعية في عمل العديد من الأعضاء الحيوية بما في ذلك جهاز تصنيع الدم.

هذه دراسة وصفية مقطعية تهدف لمعرفة التغيرات في بعض قياسات الدم لدي عمال محطات البنزين بولاية الخرطوم في الفترة من أغسطس الي ديسمبر 2015. شملت الدراسة 40 عاملا بمحطات البنزين و30 شخصا كمجموعة ضابطة مماثلين للمجموعة موضوع الدراسة في العمر. تم اخذ 2.5 مل من الدم الوريدي ووضعت في حافظات مانعة للتجلط تحتوي علي حامض الخليك الثلاثي الاميني الثنائي لتعداد الدم الكامل وحللت بجهاز (Sysmex kx 21N) وقد تم تحليل البيانات بواسطة برنامج حاسوب الحزم الاحصائية للعلوم الاجتماعية نسخة 16 عبر اختبار مستقل واختبار انوفا زي الاتجاه الواحد.

اظهرت نتائج هذه الدراسة إرتفاعا في متوسط خلايا الدم الحمراء (4.7±0.3 vs 5.02±0.65) ومادة الهيموغلوبين (13.6±0.54 vs 14.5±1.01) تركيز الهيموقلوبين في الكرية (84.5±8.2 vs 88.9±5.6) ، و متوسط تركيز الهيموغلبين في الكرية (32.4±1.4 vs 43.5±5.5) لدي العاملين بمحطات البنزين عند مقارنته بالمجموعة الضابطة. وأن مكداس الدم ومتوسط هيموغلبين كريات الدم الحمراء في المستوي الطبيعي ولا يوجد اي اختلاف في قياسها عند مقارنتها بالمجموعة الضابطة القيمة المعنوية (0.05<) وأن الصفائح الدموية قد انفضت بشكل ملحوظ عند مقارنتها بالمجموعة الضابطة (211±77.89 , 264±86.72). كما أظهرت ايضا ارتفاعا في مجموع خلايا الدم البيضاء الحمضية والقاعدية وخلايا البلعمة(0.81±0.45, 0.6±0.25) لدى عمال محطات البنزين عند مقارنتهم بالمجموعة الضابطة. وأن الصفائح الدموية القيمة المعنوية(0.000) والخلايا الليمفاوية القيمة المعنوية (0.017) تنخفض تدريجيا بزيادة عدد سنوات العمل .بينما خلايا الدم الحمراء،مجموع خلايا الدم البيضاء، مادة الهيموغلبين، متوسط هيموغلبين كريات الدم الحمراء، متوسط حجم كريات الدم الحمراء ،متوسط تركيز الهيموغلبين في الكريةو، مجموع خلايا الدم البيضاءالحمضية والقاعدية وخلايا البلعمة لا يوجد اختلاف في تعدادها بين عمال محطات الوقود والمجموعة الضابطة القيمة المعنوية (0.05<)

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Abbreviations

BFU-E	Erythroid brust forming unit
CBC	Complete blood count
CD	Cluster of differentiation
CSFs	Colony stimulating fractor
CFUGEMM	Colony-forming unit, granulocyte, erythroid, monocyte, and megakaryocyte
CFU-Baso	Colony- forming unit basophil
CFU- Eo	Colony- forming unit eosinophil
DNA	Deoxy-ribo nucleic acid
GM- CSF	Granulocyte Monocyte-colony stimulating factor
HB	Hemoglobin
HCT	Hematocrit
HiCN	Cyanomethemoglobin
IgD	Immunoglobulin D
Ig M	Immunoglobulin M
K2EDTA	Di potassium ethylenediaminetetra-acetic acid
LCD	liquid crystal displayer
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
MXD	Mixure content of monocytes, basophils,and eosinophil
NK Cell	Natural killer cell
PCV	Packed cell volume

RBCs	Red blood cells
RNA	Ribonucleic acid
SD	Standard deviation
SPSS	Statistical package for social sciences
TWA	Time-weighted average
WBCs	White blood cells

Chapter One

Introduction and literature review

1-1 Introduction

Benzene, an aromatic hydrocarbon, that is a natural component of crude oil and petroleum product, it is toxic to the blood and blood forming organs. The cells of the hematopoietic system are the most sensitive target organs. Repeated occupational exposure over long periods of time may affect several hematopoietic parameters. (Rothman *et al*., 1996).

Exposure can occur occupationally and domestically as a result of ubiquitous use of benzene-containing petroleum products, including motor fuels and solvents. (WHO 2010).

Several epidemiological studies have demonstrated harmful effects of benzene on human health (Aksoy1 985; Rothman *et al*.,1996)for example exposure has been shown to increase the risk of developing leukemia, lymphoma, a plastic anemia , pancytopenia and chromosomal aberrations (Aksoy *et al.*, 1985, 1989; WHO, 1993; and Snyder, 2000).

A complete blood count (CBC) has been recognized as an easy and readily available screening tool for assessing the hemtotoxcicity of benzene (Goldstein, 1988). We conducted this study to find out any changes in some hematological parameters among persons occupationally exposed to benzene as a first line risk group.

1-2 Literature review

1-2-1 Definition of blood:

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

1-2-2 Functions of blood: `

Normally, the Functions of blood are to deliver nutrients, hormones, and oxygen to tissues; to collect and dispose the wastes from cellular metabolism; to deliver specialized tissues for protection against the external environment; and to prevent leakage by closing holes in blood vessels. (Emmanuel *et al.,* 1993).

1-2-3 Blood components:

Its composed of two major elements, cellular component and fluid elements:

1-2-3-1 Cellular components:

A-Red blood cells (RBCs):

Are the cells responsible for carrying oxygen and carbon dioxide between the lungs and tissues via the hemoglobin content in their cytoplasm .the name of red blood cell reflect the bright red color of cell that occurs when oxygen is attached to hemoglobin . The cell is disk-shaped and biconcave .because the cell does not have a nucleus. (Emmanuel *et al*., 1993).

B-White blood cells:

Are colorless nucleated cells whose primary function is protection against invading organisms. The white blood cell performs its function in one of two ways. (Emmanuel *et al*., 1993).

- Phagocytes engulf: or phagocytize, the pathogenic organism or foreign particle. These cells have the capacity to move from the blood stream into the tissues where they are needed. Phagocytes can be identified by the histologic staining of their granules (granulocytes such as neutrophils, basophils, eosinophils) or by their nuclear characteristics (mononuclear cells in the circulation are monocytes, in tissue are macrophages). (Emmanuel *et al*., 1993).

- Immunocytes:

Are associated with humoral and cell mediated reactions of the immune system. B and T lymphocytes are immunocytes in the blood; Plasma cells are found in the bone marrow. (Emmanuel *et al.,* 1993).

- Platelets:

Are nucleated, disk-shaped cytoplasmic fragments of megakaryocytic, the precursor cells in the bone marrow. Platelets are release into the circulation to prevent leakage or bleeding caused by inherent or acquired defects in blood vessel walls. (Emmanuel *et al*., 1993).

1-2-3-2 Fluid elements:

Plasma is a fluid portion of blood in which the cellular elements are suspended and circulated throughout the body. (Serum is a clear fluid that separates from the blood up on coagulation, when all cellular elements are removed) plasma has three main components. (Emmanuel *et al*., 1993).

- **Water:**

Is the main component of blood .Almost 70%of the body water, most of which is contained in and around cells. The blood plasma maintains the water content of cells in the tissues.(Emmanuel *et al*., 1993).

- **Electrolytes:**

Electrolytes in the plasma are essential to cellular function. The important plasma electrolytes are sodium, potassium, chloride, hydrogen, magnesium, and calcium.(Emmanuel *et al.,* 1993).

- **Proteins:**

Are abundant in plasma. Although highly varied in structure, plasma proteins can be divided into three main functional classes. (Emmanuel *et al*., 1993).

(A) Coagulation proteins:

Are primarily involved in keeping the vascular system intact. Proteins that form clots (procoagulants) include components of the extrinsic and intrinsic pathways of the coagulation cascade and fibrinogen. Proteins that break down

excessive coagulation (anticoagulant) are components of the fibrinolytic system. The procoagulant and anti coagulants maintain a balance of clot formation and clot dissolution. (Emmanuel *et al*., 1993)

(B) Proteins with immunogenic functions:

Include antibodies (immunoglobulin's) and the components of the complement system. These plasma proteins are involved in defending the body against infections caused by invading organisms and against the presence of foreign antigens. (Emmanuel *et al*., 1993).

(C) Transport proteins:

Serve several functions.

(1) Osmotic pressure is maintained in the intravascular space by albumin, which prevents excessive leakage of blood fluid extracellularly. These enable the circulations of blood within the vessels and prevent edema.

(2)Binding and transporting substances to the tissues and removal of waste products and toxins in the circulations are also functions of transport proteins.

a) Transferrin is a plasma protein that binds and transports iron to the bone marrow for production of red blood cells.
b) (b)Transcobalamin is a carrier protein for vitamin B12.
c) Haptoglobin protects tissue cells from noxious substances by binding the byproduct of hemoglobin released from senescent or prematurely destroyed red blood cells and transporting by byproducts to the liver for disposal.

(3)Transportation of lipids, cholesterol, and triglycerides is carried out by lipoproteins. (Emmanuel *et al*., 1993).

1-2-4 Hematopoiesis:

Hematopoiesis is the proliferation of the progenitor cells, and their differentiation into all the cellular components of blood. (Emmanuel *et al*., 1993)

1-2-4-1 Erythropoiesis:

Erythropoiesis passes from the stem cell through the progenitor cells colony-forming unite granulocyte, erythroid, monocyte, and megakaryocyte (CFUGEMM), brust-forming unite erythroid (BFUE) and erythroid CFU(CFUE)to the first recognizable erythrocyte precursor in the bone marrow , pronormoblast. This is a large cell with dark nuclei and blue cytoplasm, a central nucleus with nucleoli and slightly clumped chromatin. The pronormoblast give rise to a series of progressively smaller normoblast by number of cell divisions. They also contain progressively more hemoglobin (which stains pinks) in the cytoplasm; the cytoplasm stains paler blue as it loses its RNA and protein synthetic apparatus while nuclear chromatin becomes more condensed. The nucleus is finally extruded from the late normoblast within the marrow and reticulocyte stage results which still contains some ribosomal RNA and is still able to synthesize hemoglobin .This cell is slightly larger than a mature red blood cell, spends 1-2 days in the marrow and also circulates in the peripheral blood for 1-2 days before maturing, mainly in the spleen, when RNA is completely lost.

A completely pink-staining mature erythrocyte result which is a non-nucleated biconcave disc. (Hoffbrand, *et al.,* 2006)

1-2-4-2 Granulo-monocytopoiesis:

In hematopoiesis, stem cells commit to differentiation along a particular pathway. The signals that induce such commitment are poorly understood but are thought to involve, at least to some extent, stromal cells of the bone marrow, which are sometimes referred to as hematopoietic microenvironment.

Hematopoietic growth factors for granulomonocytopoiesis {sometimes called colony-stimulating factors (CSFs} contribute to the maturation of committed precursors and probably also initiate their self –renewal to satisfy the next demand for that cell linage. These factors, which are produced by T cells and by endothelial and mesenchymal cells, include the following

a) Granulocyte/macrophage colony-stimulating factor (GM-CSF) stimulates production and function of neutrophils, monocytes, and eosinophils.
b) Granulocyte colony stimulating factor (GM-CSF) stimulates production and maturation of neutrophils.

c) Macrophage colony-stimulating factor (M-CSF) stimulates production and maturation of monocytes.(Emmanuel *et al*., 1993)

- **Neutrophils:**

Mature over 1-2 weeks in the bone marrow to yield fully differentiated forms.The mature neutrophil is characterized by a light orange-pink mature cytoplasm that contains granules and a nucleus of three or four segments connected by thin chromatin filaments. (Emmanuel *et al*., 1993).

- **Monocyte:**

Bone marrow monocytes give rise not only to circulating monocytes but also to an extensive network of macrophages referred to as mononuclear phagocyte system.

Monocytes vary in size from10-20 micrometer .macrophage cell size ranges up to 50 micrometer in tissue where monocytic cells may fused under condition of inflammation to form large multinucleated langerhansgaint cells.

Nuclei often are slightly intended and have reticular chromatin that is less clumped than in lymphocytes. The relatively abundant cytoplasm is gray to blue and contains small orange –red granules. Cytoplasmic vacuoles are common. (Emmanuel *et al.,* 1993).

- **Eosinophil:**

Mature eosinophils are easily recognized with cells with retractile orange-red granules and segmented nuclei on stained peripheral blood smears in contrast to neutrophils; many eosinophils exhibit a bilobed nucleus .maturation is similar to that of neutrophils. (Emmanuel *et al.,* 1993).

- **Basophil:**

Drive from granulocytic precursors and exhibit segmented nucleoli .the cytoplasm contains blue a few large granules. (Emmanuel *et al.,* 1993).

1-2-4-3 Lymphopoiesis:

Lymphoid stem cells give rise to the major functional classes of lymphocytes which morphologically resemble small lymphocytes, are derived from pluripotential hematopoietic stem cells. (Emmanuel *et al.,* 1993).

As lymphocytes differentiate from precursor into functionally mature forms, there is a complex programmed expression involving both acquisition and loss of series of surface antigens, which can be defined by monoclonal antibodies. Because antibodies from different sources often recognize identical antigens, cluster designations (CD) have been defined for most major surface determinants. (Emmanuel *et al*., 1993).

- **B cells:**

Are derived from stem cells that have undergone conditioning to establish that their progeny will produce immunoglobulins. In birds, conditioning occurs in a specific organ, the bursa of fabricius. In human this function is thought to be served by the bone marrow. A discrete series of steps lead to mature B cell capable to producing immunoglobulin. (Emmanuel *et al*., 1993).

- **T cells:**

Are derived from stem cells that undergo maturation in the thymus, where T cells acquired the capacity of specific immunoglobulin roles. (Emmanuel *et al*., 1993).

- **Lymphocytes morphology**:

Are small (6-10micrometer) to medium-sized (10-15micrometer) with relatively high nuclear –cytoplasm ratios, scant to moderate light blue cytoplasm, and relatively few cellular organelles. Some lymphocytes, particularly NK cells, display scattered prominent cytoplasmic granule . (Emmanuel *et al*., 1993).

- **Plasma cell:**

Have eccentric nuclei , often with condensed chromatin along the nuclear rim to form a “clock-face” pattern and basophilic cytoplasm reflecting abundant RNA .A perinuclear cytoplasmic clear zone, reflects an active Golgi complex. (Emmanuel *et al*., 1993).

1-2-4 Thrombopoiesis:

Platelets are produced by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body. Giving rise to 1000-5000 platelets. The time

interval from differentiation of the human stem cell to the production of the platelets averages approximately 10 days. (Hoffbrand *et al.*, 2006).

1-2-5 Complete blood count:

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

This test is of great value and it's aimed to:

-Screening test to check some blood disorders "Anemia, Infection, and inflammation"

- Use to determine the general health status of people.

-Use to monitoring and following up the treatment and drugs effects. (Dace and Lewis 2006).

1-2-5-1 Hemoglobin:

Is the main solute in the erythrocyte and its properties affect the red cell adaptability in size, shape, flexibility, flow, and function. Hemoglobin takes up oxygen from pulmonary circulation and delivers it to the tissues. (Emmanuel *et al*., 1993).

1-2-5-2 Red cell indices:

Are measurements that indicate the size and hemoglobin content of red cells. These values can be calculated quantitively from the hemoglobin concentration, red blood cell counts, and packed red cell volume. (Emmanuel *et al.*, 1993)

- **MCV:**

Refers to the average volume of the individual red cell. The MCV is expressed in cubic micrometers (μm^3) per red cell or femtoliters (fl). (Emmanuel *et al.*, 1993)

- **MCH:**

Refers to hemoglobin content per red cell. The MCH is expressed in pictogram (pg) per red cell. (Emmanuel *et al.*, 1993)

- **MCHC:**

Refers to the hemoglobin concentration of red cells. The MCHC is expressed in grams per deciliter (g/dl) per red cell. (Emmanuel *et al.,* 1993)

1-2-6 Blood disorders:

1-2-6-1 Anemia

Is defined as a reduction in the hemoglobin concentration in the blood .typically values will be less than 13.5g/dl in adult males and less than 11.5g/dl in adult females. From the age of 2 years to puberty, less than 11.0g/dl indicates anemia. As newborn infants has a high hemoglobin level 14.0g/dl is taken as a lower limit at birth. (Hoffbrand *et al.,* 2006).

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 hemorrhage or chronically through low-volume loss), excessive blood cell destruction (hemolysis) o deficient red blood cell production (ineffective hematopoiesis). (Dace and lewis, 2006)

1-2-6-2 Leukopenia :

leucopenia (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. (Dace and lewis 2006).

1-2-6-3 Thrombocytopenia:

The terms thrombocytopenia and thrombopenia, refer to a relative decrease of platelets in blood. (Dace and lewis2006).

1-2-6-4 Pancytopenia:

Pancytopenia describes a reduction in the blood count of all the major cell lines red cells, white cells, and platelets. (Hoffbrand *et al.*,2006).

1-2-6-5 Leukemia :

The leukemias are a group of disorders characterized by the accumulation of malignant white blood cells in the bone marrow and blood. (Hoffbrand *et al.*, 2006).

1-2-6-6 Lymphoma:

Lymphomas are group of diseases caused by malignant lymphocytes that accumulate in lymph nodes and cause the characteristic clinical features of lymphadenopathy. Occasionally, they may spill over into blood (leukemic phase) or infiltrate organs outside the lymphoid tissue. (Hoffbrand *et al.*, 2006).

1-3 Benzene:

1-3-1 Definition:

Benzene is a hydrocarbon chemical consisting of six atoms arranged in a ring structure .At normal ambient temperatures; it is a liquid, which evaporates rapidly at room temperature and is highly flammable. It has a characteristic of aromatic odor and is slightly soluble in water (1.5g/liter) but miscible with most other organic solvents. (Bruce *et al.;* 2005).

1-3-2 Physical and chemical properties: (HSDB, 2007)

Description	clear, colorless liquid
Molecular formula	C6H6
Molecular weight	78.1 g/mol
Density and specific gravity	0.8787 at 15C/4C
Boiling point	80.1C
Melting point	5.5C
Vapor pressure	94.8 mmHg@ 25C (0.125atm)
Flashpoint 5.5C	-11C
Solubility	miscible with ethanol, chloroform, ether, carbon disulfide, acetone , oils, and glacial acetic acids; slightly soluble in water (1.790 mg/l at 25C°)
Odor threshold	4.68 ppm (15.3mg/m3)
Oder description	aromatic odor (sweet); gasoline-like odor
Metabolites	hydroquinone, benzoquinone, catechol,phenol
Conversion factor	1 ppm= 3.26 mg/m3

1-3-3 Sources of exposure to benzene:

Benzene is a highly volatile, and most exposure is through inhalation. However, dermal absorption of benzene is not extensive, as it evaporates quickly due to high vapor pressure.

1-3-3-1 Industrial processes:

As benzene is occur naturally in crude petroleum at levels up to 4g/l , human activities include processing of petroleum products, coking of coal , production of toluene, xylene and other aromatic compounds , and use in industrial and consumer products , as a chemical intermediate and as a component of petrol (gasoline) and oils. The presence of benzene in petrol and as a widely used industrial solvents can result in significant occupational exposure and wide spread emissions to the environment. Automobile exhaust accounts for a largest source of benzene in general environment. Off-gassing from building materials and structural fires lead to increased atmospheric benzene levels. Industrial discharge, landfill leachate and disposal of benzene- containing waste are also sources of exposure. (IPCS 1993).

1-3-3-2 Indoor residential air:

Benzene has been detected at high levels in indoor air. Although some of this exposure might be from building materials (paints, adhesives, etc.), most is from cigarette smoke in both homes and public spaces. Levels are increased in homes with attached garages than in those with detached garages. Levels are increased in homes close to petrol filling stations. (IPAC1993).

1-3-3-3 Inside vehicles:

Benzene has been measured in air inside vehicles at levels higher than those in residential air, but substantially lower than those at petrol filling stations (WHO 2000).

1-3-3-4 Food and water:

Waterborne and foodborne benzenes contribute only a small percentage of total daily intakes in non-smoking adults. (IPCS 1993).

1-3-4 Metabolism:

The liver is the major site of metabolism of benzene (Snyder and Hedli1996).

Benzene is detoxified in two phases. During phase I, benzene is oxidized by cytochrome P450 2E1, forming benzene oxide, an electrophilic reactive intermediate. Subsequently, benzene oxide is metabolized by three pathways (Snyder and Hedli1996):

1) Rearrangement non-enzymatically to form phenol;

2) Hydration by epoxide hydrolase to 1,2-benzene dihydrodiol, which in turn can be oxidized by dihydrodiol dehydrogenase to form catechol; and
3) Glutathione conjugation with glutathione *S*-transferase to form a premercapturic acid, which is converted to phenylmercapturic acid.

Phenol can undergo subsequent hydroxylation to hydroquinone, with the consecutive production of *p*-benzoquinone and 1, 2, 4-trihydroxybenzene. Alternatively, phenol can be hydroxylated to catechol, which is converted to *o*benzoquinone.

The benzene ring can also be opened either at the benzene oxide or oxepin stage, forming muconaldehyde.

All these metabolites can then undergo a phase II metabolism, leading to excretion of glucuronide and sulfate conjugates, mercapturic acid ring-opened metabolites and DNA adducts in urine (Snyder and Hedli1996).

1-3-5 Adverse health effects:

1-3-5-1 Acute effects:

Acute occupational exposure to benzene may cause narcosis: headache, dizziness, drowsiness, confusion, tremors, and loss of consciousness (IPCS 1993).

1-3-5-2 Long term effects:

Benzene has been classified by international agency for research on cancer as a human carcinogen, and the carcinogenic effect may occur even at low-level exposure (IARC, 1982).

Chronic exposure to benzene can reduce the production of both red and white blood cells from bone marrow in humans, resulting in a plastic anemia. (IPCS1993).

Benzene exposure has been shown to increase the risk of developing anemia, pancytopenia, and chromosomal aberrations (Aksoy, 1985, 1989; WHO, 1993; Synder, 2000).

There is also evidence suggesting that the immune system can be affected by benzene exposure (Macedo *et al.*, 2007).

It has been established that reactive metabolite for the immune toxicity associated with benzene exposure, including suppression of B lymphocytes (Pyatt *et al.*, 1998).

1-4 Previous studies:

A retrospective longitudinal study correlated average benzene exposure with total white blood cell counts in cohort of 459 pliofilm rubber workers in Ohio (Kipen *et al.,* 1988) The authors found a significant ($P<0.016$) negative correlations between average workplace benzene concentrations and white blood cell counts for the years 1940-1948.

An examination of 32 patients, who were chronically exposed to benzene vapors ranging from 150- 650 ppm for four months to15 years, showed that pancytopenia occur in 28 cases. Bone marrow punctures revealed variable hematopoietic lesions, ranging from a cellularity to hyper cellularity (Aksoy *et al* 1972).

Collins *et al* (1997) used routine data from Monsanto's medical industrial hygiene system and study 387 workers with daily 8 hours- weighted exposure (TWA) averaging 0.55ppm for evaluation of lymphopenia among workers with low low-level benzene exposure and they reported lymphopenia is not a risk factor.

Violante *et al*., 2003 found no significant association between hematological profile and benzene exposure.

Sara and Mahadi (2015) studied 100 fuel station workers, chronically exposed to petroleum fumes in Khartoum city and compared them with 50 normal non exposed subjects as control, and reported that exposure to petroleum fumes result in reduction of multiple blood cell lineages, including WBCs, neutrophils, and platelets .and found no significant differences were observed in HB level, HCT, and RBCs between cases and controls.

1-5 Rationale

Occupational benzene exposure is one of the greatest public health problems particularly in developing countries where the majority of workers are neglecting or lacking of the primary protective safety measures beside poor medical surveillance system which make them more susceptible to toxic benzene fumes.

Exposure to benzene fumes have been reported to have toxic effects on various organs and systems including hematopoietic system and those occupationally exposed are likely to be more affected. The current study intended to find out any changes in some hematological parameters among benzene station workers as a first line risk group.

1-6 Objectives

1-6-1 General objectives:

The overall aim of the study was to Assessment of changes in some hematological parameters among fuel station workers in Khartoum State.

1-6-2 Specific objectives

- To measure HB, PCV, MCH, MCHC, and RBCs.
- To measure WBCs count and, differential leukocyte count.
- To determine Platelets number.
- To assess the association of work duration in benzene stations and its consequences on hematological parameters.

Chapter Two

Materials and Methods

2-1 Study design:

It was descriptive cross sectional study carried out in Khartoum State from August to November 2015 aimed to investigate some hematological changes in benzene station workers.

2-2 Study population:

We investigate 40 randomly selected filling-pump workers at seventeen petroleum stations located in Khartoum having mean age of (34years) with average serving of (8 years) in average daily work of about (6 hours), and 30 control subjects selected from general population who has no history of being worked at petrol stations .and matching the study group in age.

2-3 Inclusion criteria:

All benzene station workers who agreed to participate in the study.

2-4 Exclusion criteria:

- Subject suffering from significant cardiovascular disorders
- Subject with chronic diseases particularly renal diseases and respiratory diseases.
- Subjects on medications which may affect the results of study.

2-5 Sampling technique:

Simple random technique.

2-6 Sample collection:

- Blood collection was performed as described by (Dace and lewis,2006)
- Blood was withdrawn from anntecubital vein by means of a syringe. The skin was cleaned with 70% alcohol (isopropanol) and allowed to dry spontaneously before being punctured. About 2.5 ml blood sample from each individual was drowning on K2EDTA anticoagulant tube for complete blood count.

2-7 Materials required:

- Blood analyzer.
- EDTA blood containers.
- Syringes.
- Tourniquet.
- 70% isopropyl alcohol swab.
- Cellulose pads.

2-8 Blood analyzer:

2-8-1 Principle of the blood analyzer (Sysmex KX 21N):

Measurement of blood cells (RBCs, WBCs, and Platelets) and HB concentration obtained by aspiration of small volume of well mixed K2EDTA blood by sample probe and mixed with isotonic diluents in nebulizer. Diluted mixture aspiration delivered to RBCs aperture path for providing information about RBCs and platelets based on size. [articles of 2 to 20fl counted platelets above 36 fl count as Red cells. some portion of aspirated mixture induced into WBCs path in which hemolytic reagent (Stromatolyzer) added automatically to measure HB concentration in a build colorimeter .based on cynomethemoglobin (HiCN) blood cell count and size information generated in triplicate pulses according to electronic conductivity and translated into digital number using in build calculator programmed and designed for RBCs. WBCs count hence three values were directly measured (RBCs, WBCs, HB)and displayed on (LCD) other values of red cell indices . platelet leukocyte differential and absolute count calculated from given information and automated histogram . The result is printed out according to setting mode. (Sysmex manual).

2-8-2 Procedures:

The instrument was checked up for the sufficient of the solutions (all pack stomatolyser), also checked electrical power supply machine has full battery and earthed connected then power key was pressed on.

Sample was well mixed and entered to probe then the start switch was displayed analyzing the aspirated sample. The results were printed out within 30 seconds.

2-8-3 Quality control:

Quality control was intended to ensure that measurements are sufficiently precise day by day or batch by batch within established limits. Results on patient samples were not issued until it was clear from the control data that there had been no significant problem in the analytic procedure.

Control sample was tested at intervals a alongside the routine specimens, and plotted the results on a Levey-jenning control chart. This linear graph showing the mean and limits of standard deviations (SD) at 1SD and 2SD.

The results of sequential (daily) measurements were plotted on the graph, when system was in good control, not more than 1 in 20 measurements should fall outside 2SD. When two or more consecutive measurements were outside this limits there is likely to have been a random error, whereas several consecutive values within 2SD, but all on one side of the mean, indicate a consistent bias. A wildly deviant result outside 3SD may occur as a result of a gross error (‘blunder’).

2-9 Data collection:

Data for this study was collected by direct interview of the participants after their agreement, using pre designated Questionnaire.

2-10 Ethical consideration:

Official agreement from the petrol station manager preceded the conduction of the study. An informed consent for participants in the study was obtained including an explanation about purpose of the study and assurance about the confidentiality of the information and that the participation was optional.

2-11 Data analysis:

All the data was presented in tables as mean± SD. Data was analyzed using Statistical Package for Social Sciences (SPSS version 16) by student t test and one way ANOVA.

Chapter Three

Results

The result of the present study revealed a statistically significant increase in the mean of RBCs $\times 10^6/\mu l$ (5.02±0.65 vs 4.7±0.3), HB g/dl (14.5±1.01 vs 13.6±0.54), MCV fl (84.5±8.2 vs 88.9±5.6), and MCHC g/dl (43.5±5.5 vs 32.4±1.4) of the benzene station workers when compared to control subjects P.value (≤0.05). Table (3-1)

HCT % and MCH pg were within normal ranges and did not vary significantly between two groups P.value (<0.05) table (3-1).

Platelets count $\times 10^3/\mu l$ were showed significant decrease in studied group (211±77.89 vs 211±77.8) Table (3-1)

Table (3-2)

show significant increase in MXD $\times 10^3/\mu l$ in benzene exposed group (0.81±0.45 vs 0.6±0.25) as compared to the control P.value (<0.05), whereas WBCs $\times 10^3/\mu l$, lymphocytes $\times 10^3/\mu l$ and neutrophils $\times 10^3/\mu l$ were within normal limits and did not vary significantly between benzene station workers and the control P.value (<0.05).

Table (3-3)

Platelets $\times 10^3/\mu l$ P.value (0.000) and lymphocytes $\times 10^3/\mu l$ P.value (0.017) were showed significant decrease among studied group as duration of work increased.

WBCs $\times 10^3/\mu l$, RBCs $\times 10^6/\mu l$, HBG g/dl, HCT %, MCV fl, MCH pg, MCHC g/dl, MXD $\times 10^3/\mu l$, and neutrophils $\times 10^3/\mu l$ were within normal limits and did not significant change with regard to the years of exposure P.value (<0.05).

Erythrocyte Series:

Table (3-1)

Mean erythrocytic and thrombocytic series on benzene station workers and control (mean±SD):

Parameter	**Workers (mean ± SD)**	**Control (mean ± SD)**	**P.value**
RBCs $\times 10^6/\mu l$	5.02±0.62	4.7 ± 0.3	0.031
HBG g/dl	14.5 ± 1.01	13.6± 0.54	0.000
HCT %	43.1 ± 5.7	42.1± 1.6	0.367
MCV fl	84.5 ± 8.2	88.9 ± 1.9	0.014
MCH pg	29.7 ± 4.6	28.9 ± 1.9	0.302
MCHC g/dl	34.5 ± 5.5	32.4 ± 1.4	0.042
PLTs $\times 10^3/\mu l$	211 ± 77.89	264 ± 86.72	0.011

Significance level at $P \leq 0.05$

Table (3-2)

Mean leukocyte Series on benzene station workers and control (mean ±SD)

Parameter	Workers (mean ± SD)	Control (mean ± SD)	P.value
WBCs$\times 10^3/\mu l$	5.9 ± 1.3	5.8 ± 1.4	0.758
Lymph $\times 10^3/\mu l$	2.11 ± 0.66	2.2 ± 0.89	0.392
MXD$\times 10^3/\mu l$	0.81 ± 0.45	0.6 ± 0.25	0.015
Neutrophil $\times 10^3/\mu l$	2.5 ± 1.36	2.9 ± 1.02	0.146

Significance level at $P \leq 0.05$

Table (3-3)

Some hematological parameters of benzene station workers according to years of work (mean ±SD)

Parameter	Less than 5 years (mean±SD)	6-10 years (mean±SD)	More than 10 years (mean±SD)	P.value
WBCs $\times 10^3/\mu l$	6.02±1.15	6.07±0.84	5.34±1.43	0.312
RBCs $\times 10^6/\mu l$	5.07±0.45	5.07±0.44	4.88±0.6	0.631
HBG g/dl	14.23±1.18	13.9±1.3	14.4±1.1	0.679
HCT %	43.4±3.6	43.3±3.2	42.3±5.5	0.879
MCV fl	85.7±5	85.5±4.8	86.5±3.6	0.839
MCH pg	28.1±1.9	27.6±1.5	30.05±4.8	0.250
MCHC g/dl	28.1±1.1	27.6±1.5	24.7±6.07	0.355
PLTs $\times 10^3/\mu l$	272.5±65.06	233.7±36.33	151.3±37.24	0.000
LYM $\times 10^3/\mu l$	2.5±0.5	2.3±0.58	1.82±0.54	0.017
MXD $\times 10^3/\mu l$	0.76±0.5	0.86±0.48	0.72±0.31	0.770
NEUT $\times 10^3/\mu l$	1.85±1.3	2.9±0.75	2.5±1.3	0.215

Significance level at $P \leq 0.05$

Chapter Four

Discussion, Conclusion and Recommendations

4-1 Discussion

Khartoum city experience serious and continuously increasing environmental and occupational problems. Human exposure to benzene has been associated with a range of acute and long-term adverse health effects and diseases, including cancer and aplastic anemia. (WHO, 2010).Exposure can occur occupationally and domestically as a result of ubiquitous use of benzene-containing petroleum products, including motor fuels and solvents (WHO 2010).

The present study indicate that RBCs, HB, MCV, MCHC are significantly higher among benzene station workers when compared to control subjects P.value (<0.05) . The lower values in RBCs, HB, MCV, and MCHC have been found by Rothman *et al* (1996) in a cross sectional study of 44 workers heavily exposed to benzene and as an 8-hrs time weighted average and 44 age and gender-matched unexposed controls from Shanghai ,China. Our results are on line with Uzma, *et al* (2008) and were also supported by the study conducted by Erslev *et al* (1990) They showed that exposure to carbon monoxide which emitted mainly by internal combustion engines of motor vehicles readily enters the blood through the respiratory system and binds more firmly to hemoglobin than oxygen, forming carboxy hemoglobin and seriously interfering with blood oxygen transport capability, which ultimately leadsing to hypoxic hypoxia, Tissue hypoxia is the most potent stimulus for erythropoises so it leads to stimulation of erythropoietin – a factor which stimulate erythropoises which ultimately leads to production of more RBCs and hemoglobin in circulating blood .However duration of exposure to benzene fumes , nutritional habits and differences in techniques through which the blood had been analyzed may account for deviations. In contrast; the counts of MCH, and HCT, were within the normal ranges and had no significant differences with those of control Pesatori *et al* (2009) and Collins *et al* (1997) found similar result .another study in Nigeria on fuel station workers show reduction in red blood cell indices in exposed individuals (Okoro *et al.,* 2006).

Platelets counts were decreased in benzene station workers as compared to control group. This finding was consistent with Rothman *et al,* (1996). Pestori *et al* (2009) who reported that No effects was observed on platelets count and most hematological outcomes.

No changes were observed on WBCs, lymphocytes, and neutrophils counts in benzene station workers in comparison to controls. This result is in agreement with some studies that reported, no decrease was found in WBCs number and no increase in prevalence of lymphopenia and other measures of hematotoxcicity among workers exposed to low levels of benzene (Khuder *et al*, 1999) and Pesatori *et al* (2009). QU *et al* (2002) examined hematological changes among Chinese workers with abroad range of benzene exposure, they found a significant decrease in WBCs, neutrophils, and lymphocytes counts as compared to control. Reason for deviation from this result might be due to toxicant concentrations in work place and duration of exposure to benzene fumes.

The absolute content of mixture monocytes, basophilis, and eosinophils (MXD) show significant increase values among workers. No reported data was found on this parameter, since most studies measure such cells separately, However eosinophilia and basophilia were reported by Aksoy *et al* (1971), monocytosis reported by Ray *et al* (2007), a drop in eosinophil count reported by Tunsaringkaran *et.,al* (2013)

As the work duration progresses; results indicate that there was a significant decrease in platelets number, and lymphocytes Hameed *et al* (2009) in agreement with such findings. No changes were found on the other parameters with duration of work progresses in benzene exposed workers. Reduction on other blood cells RBCs, whole WBCs were reported by Hameed *et al* (2009).

Although the overall results of the current study are generally consistent with many of previous studies it might be more reliable if conducted on a large population.

4-2 Conclusion

From the result of this study, It is concluded that inhalation of benzene fumes result in

- increase red blood cell count and its dependent indices (HCT, HB, MCV, MCHC
- depression of platelets count
- An increase of MXD mixture content of eosinophils, basophils, and monocytes among workers.
- Gradual decrease of platelets as well as lymphocytes as the duration of work progresses.

Such results suggest that these workers are susceptible to have serious hematological changes.

4-3 Recommendations:

1. A clear educational and protective policy should be implemented for occupationally exposed workers.
2. Monitoring blood count at regular interval to detect preclinical abnormalities.
3. Further research is recommended by using more sensitive procedures.
4. Need to study more deeply on large populations to check the effects of exposure to benzene fumes.

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Appendix No (1)

Sudan University OF Science and Technology

College Of Graduate Studies

Questionnaire

Serial No: ……..……

Date: / /2015

Name: …….

Age (years): ……..

Education:

Illiterate ( ) Primary ( ) Sec. School ( )

University ( )

How long have you worked in the petrol station? (years of work)……..

Smoking:

Yes ( ) No ( )

Alcohol consumption:

Yes ( ) No ( )

Are you suffering from any diseases?

Yes ( ) No ( )

Medications: ……..……

Do you drink milk frequently?

Yes ( ) No ( )

Do you use protective safety clothes, gloves, face mask and glasses?

…… If no, why? …….

Test and result:

HB: …….. HCT: …… RBCs: ……. MCV: …….

MCH: ……. WBCs: ……. MCHC: …… MXD: …….

NEUT: ……. PLT: …….

Appendix No (2)

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا – برنامج الماجستير – مختبرات طبية

تخصص علم الدم ومبحث المناعة

براءة أخلاقية

الإسم: .....

سوف يتم أخذ عينة من الدم (3 مل) من الوريد بواسطة حقنة طعن وذلك بعد مسح منطقة أخذ العينة بواسطة المطهر، كل الأدوات المستخدمة لأخذ العينة معقمة ومتبع فيها كل وسائل السلامة المعملي وأنا أقر بأن هذه العينات يتم تحليلها فقط لطلب البحث ، وأن جميع العينات وكافة البيانات الخاصة بالمشارك سرية – لا يجوز الإطلاع عليها إلا بعد موافقة المشارك شخصيا.

أوافق أنا المذكور أعلاه على أخذ عينة لإجراء الدراسة.

الإمضاء: .....

التاريخ: .....

Appendix (3)

Sysmex Kx21N

Appendix (4)

Benzene Station Worker

Appendix (5)

Normal values

Hoffbrand *et al., 2005*

Parameter	**Normal values**
Hemoglobin g/dl	13.5- 17.5
Red cells $\times 10^5/\mu l$	4.5-6.5
HCT %	40-52
MCV fl	80-90
MCH pg	27-34
MCHC g/dl	20-35
Total WBCs $\times 10^3/\mu l$	4.0-11.0
Neutrophils $\times 10^3/\mu l$	2.5-7.5
Lymphocytes $\times 10^3/\mu l$	1.5-3.5
Platelets $\times 10^3/\mu l$	150-400