Sudan University of Science and Technology

College of Graduate Studies

Estimation of Complete Blood Cells Count in Sudanese Cigarette Smokers in Khartoum North

قياس تعداد الدم الكامل لمدخني السجائر السودانيين في الخرطوم بحري

A dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc Degree in Medical Laboratory Science (Hematology and Immunohematology)

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الآية

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صدق الله العظيم

سورة العلق ﴿2,1﴾

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Dedication

I dedicate this work to my father, mother, husband, brothers, sisters and my dearest daughter Fatima. To all who contributed and helped me in accomplishing this work.

Acknowledgement

First and for most thanks to Allah who gave me strengths to carry this work. I am indebted to my supervisor **Prof: Shadia Abdalatti Omer** who guided the work successfully through all its steps. I wish to express my sincere gratitude and thanks to all the participants' we included in this work. I also thank all my family for their continuous support.

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Abbreviations

COPD	Chronic Obstructive Pulmonary Disease
CBC	Complete Blood Count
HB	Hemoglobin
HCT	Hematocrit
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
Pct.	Platelet crit
MPV	Mean Platelet Volume
PCV	Packed Cell Volume
PDW	Platelet Distribution Width
P-LCR	Platelet Large Cell Ratio
RBCs	Red Blood Cells
RDWCV	Red Cell Distribution Width Coefficient Variation
RDWSD	Red Cell Distribution Width Standard Deviation
WBCs	White Blood Cells
HiCN	Cyanomethemoglobin
EDTA	Ethyline Diamine Tetra Acidic acid
ALC	Absolute Lymphocyte Count
ANC	Absolut Neutrophil Count
CD	Cluster Differentiation
MHC	Major Histocompatibility complex
CO	Carbon Monoxide
ADP	Adenosine Di Phosphate
SPSS	Statistical Package for Social Science

Abstract

This is an analytical case control study conducted at Khartoum North during the period from September 2015 to May 2016 to investigate the effect of cigarette smoking on the complete blood cell count (CBC) of healthy Sudanese cigarette smokers.

Ninety adult healthy cigarette smokers and 70 healthy non cigarette smokers were enrolled in the study after a written consent had been obtained from them. The smokers were divided into groups ;according to their ages, the number of cigarettes consumed per day and according to the duration of smoking. Venous blood of 2.5 ml was collected in EDTA containers and CBC was determined using automated hematology analyzer (Sysmex).

Cigarette smoker showed significantly higher values than the non-smokers for:

RBC (5.16±0.61 vs.4.91±0.85×10^{12}/l), hemoglobin concentration (HB) (15.03±1.21 vs.13.92±1.23g/dl), HCT (46.42±8.34 vs.41.63±5.96 %), MCV (88.44±6.51 vs.84.79±5.76 fl), MCH (29.16±2.78 vs.27.99±2.24 pg) RDWSD (46.98±5.21 vs.43.95±3.89 fl) respectively.

Cigarette smokers recorded significantly (P≤0.05) higher values than the non-smokers with regard to TWBCs (7.16±2.41 vs.6.03±2.05 ×10^{9} /l), absolute neutrophils count (4.28±2.11vs.3.27±1.60/µl) and absolute monocytes count (0.62±0.22 vs.0.41±0.19/µl).

Smoking significantly (P≥0.05) reduced platelets count (212.30±55.71 vs. 238.23±36.89×10^{9}/l).

Age group more than 50 years showed significantly low HB concentration (13.15±0.21g/dl), while the group of less than 20 years age recorded the highest TWBCs count (8.64±3.07×10^{9}/l) and absolute neutrophils count (5.17±1.86/µl).

The group who consumed (16 – 20 cigarettes per day) recorded a significant increase in RDWCV (15.00±1.91%).

The group with smoking duration of (2 –7 years) showed significantly low HB concentration (14.91±1.35g/dl) while the group of the smoking duration (16 – 21years) showed a significant increase on HCT values (47.15±2.86%).

It is concluded that smoking caused alterations on red blood cells, hemoglobin concentrations, hematocirit, mean cell volume, mean cell hemoglobin,red cell distribution width, total white blood cell count,absolute neutrophils count,absolute monocytes counts and platelets count.

المستخلص

هذه الدراسة أجريت بطريقة الحالة الإفرادية المقترنة بحالة ضابطة لقياس صورة الدم الكاملة عند مدخنين السجائر السودانيين (من سبتمبر 2015 الى مايو 2016) في الخرطوم بحري.

تمت مقارنة تسعين عينة جمعت من المدخنين مع سبعين عينة لغير المدخنين بعد اخطارهم بهذه الدراسة واهميتها وبعد موافقتهم على المشاركة، صنف المدخنين حسب العمر وعدد السجائر المستهلكة في اليوم ثم علي حسب فترة التدخين.تم اخذ 2.5ملي لتر عينة دم وريدية من كل متبرع ووضعت في إناء بلاستيكي يحتوى على مانع تجلط (k^2EDTA) وتم اختبارها لقياس صورة الدم الكاملة باستخدام جهاز تحليل الدم الآلي (Sysmex KX21N).

استنتجت من هذه الدراسة الآتي معدلات الفحوصات عند المدخنين مقارنة مع غير المدخنين هنالك ارتفاع ذو دلالة احصائية في:

كريات الدم الحمراء ($5.16\pm0.61\times10^{12}$/l) مقارنة مع(4.91 ± 0.85)، الخضاب (15.03 ± 1.21مقارنة مع 13.92 ± 1.23g/dl)، الدم المكدس (46.42 ± 8.34% مقارنة مع41.63 ± 5.96%)، متوسط حجم الخلية (88.44 ± 6.51fl مقارنة مع84.79 ± 5.76)، متوسط حجم الخضاب في الخلية (29.16 ± 2.78 Pg مقارنة مع 27.99 ± 2.24).التشتت في كريات الدم الحمراء (46.98 ± 5.21 vs.43.95 ± 3.89 fl) على التوالي.

كريات الدم البيضاء اظهرت ارتفاع ذو دلالة احصائية ($7.16\pm2.41\times10^9$/l) مقارنة مع 6.03 ± 2.05)، العدد المطلق للخلايا وحيدة الخلية (0.62 ± 0.22/µl) مقارنة مع 0.41 ± 0.19)

الصفائح الدموية اظهرت انخفاض ذو دلالة احصائية ($212.30\pm55.71\times10^9$/l) مقارنة مع 238.23 ± 36.89).

الفروقات في الاعمار في المجموعة أكثر من خمسين سنة اظهرت انخفاض في تركيز الخضاب (13.15 ± 0.21g/dl) اما مجموعة الاعمار اقل من عشرين سنة اظهرت زيادة في عدد خلايا الدم البيضاء ($8.64\pm3.0\times10^9$/l)والعدد المطلق للخلايا العدلة (8.64 ± 3.07/µl).

عدد السجائر المستهلكة في اليوم في المجموعة التي تدخن من (16-20) سيجارة في اليوم اظهرت ارتفاع في تشتت خلايا الدم الحمراء .(15.00 ± 1.91%).

مدة التدخين بالسنين في المجموعة التي تدخن (2-7) سنوات اثرت على تركيز الخضاب بالنقصان (14.91 ± 1.35g/dl) اما المجموعة التي تدخن (16-21) سنة اظهرت ارتفاع في الدم المكدس (47.15 ± 2.86%).

استنتج من هذه الدراسة ان التدخين يسبب تغيير في عدد كريات الدم الحمراء,تركيز الخضاب,الدم المكدس,متوسط حجم الخلية,حجم الخضاب في الخلية,التشتت في خلايا الدم الحمراء,عدد كريات الدم البيضاء,العدد المطلق للخلايا العدلة,العدد المطلق لوحيدات الخلية وعدد الصفائح الدموية.

Chapter One

Introduction and Literature Review

1.1 Introduction

Smoking is a process in which tobacco is burnt and smoke is inhaled by different ways. It gives sense of pleasure and satisfaction to the smoker. It is a complex external and internal stimulus consisting of visual, tactile, mechanical (mouth movement), gustatory, olfactory and irritating factors. Cigarette smoking is one of the major leading causes of death and is an essential public health challenge. It has both acute and chronic effects on hematological parameters (Kume *et al.*, 2009) .Cigarette smoke contain more than 4000 chemical, and a cigarette smoker is exposed to a number of harmful substances including nicotine, free radicals, carbon monoxide and other gaseous products. Nicotine induces pleasure and reduces stress and anxiety. Smokers use it to modulate levels of arousal and to control mood (Gitte 2011). Paradoxically, while nicotine is a stimulant drug, effects of both stimulation and relaxation may be felt. The mental and physical state of the smoker, and the situation in which smoking occurs, can influence the way in which a particular cigarette will affect psychological perceptions. The addictive effect of nicotine is linked to its capacity to trigger the release of dopamine - a chemical in the brain that is associated with feelings of pleasure. Smoking harms nearly every organ of the body and dramatically reduces both quality of life and life expectancy. It causes lung cancer, respiratory disease and heart disease as well as numerous cancers in other organs including lip, mouth, throat, bladder, kidney, stomach, liver and cervix (Wannamethee *et al.*, 2005).

1.2 Literature review

1.2.1 Blood

It is a body fluid that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells .It is composed of blood cells suspended in blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains dissipated proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood. Blood performs many important functions within the body such as supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells),supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids),removal of waste such as carbon dioxide, urea, and lactic acid.Immunological functions including circulation of white blood cells, and detection of foreign material by antibodies, coagulation, the response to a broken blood vessel, the conversion of blood from a liquid to a semisolid gel to stop bleeding, messenger functions, including the transport hormones and the signaling of tissue damage, regulation of core body temperature and it has hydraulic functions(Dacie and Lewis 2011).

1.2.2 Blood constituents

Blood accounts for 7% of the human body weight, with an average density around 1060 kg/m3. The average adult has a blood volume of roughly 5 liters which is composed of: cells and plasma (About 55% of blood is blood plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color. The blood plasma volume totals of 2.7 – 3.0 liters (2.8 – 3.2 quarts) in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other

materials. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid. (Bruce *et al*.,2002).

The cells are:

1.2.2.1 Red blood cells

The formation of red blood cells is called erythropoiesis. Red cells are produced by proliferation and differentiation of a precursor in the bone marrow erythroblasts. Also known as normoblasts, during the course of differentiation the size of erythroblast progressively decreases, and the character of the nucleus and cytoplasm changes, hemoglobin becomes the predominant protein in the cytoplasm .Red blood cells contain the blood's hemoglobin and distribute oxygen. Mature red blood cells lack a nucleus and organelles. The red blood cells (together with endothelial vessel cells and other cells) are also marked by glycoprotein. Red cells normally enter the blood at the stage of the reticulocyte or of the mature erythrocyte, and remain within the vascular compartment during their lifespan of approximately 120 days. It is biconcave disc (7–8) μm diameter. The normal range of erythrocytes is 4.7 to 6.1 million (male), 4.2 to 5.4 million (female) (Robert *et al.*, 2006).

1.2.2.1.1 Hemoglobin

Hemoglobin is defined as a special intracellular protein found in the red cell which is responsible for gaseous exchange. It consists of four polypeptide chain α and β each with its own haem group. The molecular weight of HB is 68000 Dalton. Each Red cell contains approximately 640 million hemoglobin molecules (Hoff brand *et al.*, 2006).

In vertebrates and other hemoglobin-using creatures, arterial blood and capillary blood are bright red, as oxygen imparts a strong red color to the

haem group. Deoxygenated blood is a darker shade of red; this is present in veins, and can be seen during blood donation and when venous blood samples are taken. (Dacie and Lewis, 2011).

1.2.2.1.2 PCV

The proportion of blood occupied by red blood cells is referred to as the haematocrit. It is normally 45% for men and 40% for women. It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count. Because the purpose of red blood cells is to transfer oxygen from the lungs to body tissues, a blood sample's haematocrit—the red blood cell volume percentage—can become a point of reference of its capability of delivering oxygen. (Poli, 2004).

1.2.2.1.3 Red cell indices

The red cell indices provide information concerning the size and hemoglobin content of the red cell by providing the MCV, MCH, MCHC and the MCV is one of the most stable parameter in the CBC, read directly by instrumentation method (Ciesla, 2007).

Red cell distribution width (RDW):

It is the percentage of the measurements of the red cell volume. The RDW is derived from pulse height analysis and can be expressed either as the standard deviation (SD) in fl or as the coefficient of variation (CV).

The RDW SD is measured by calculating the width in fl at the 20% height level of the red cell size distribution histogram and the RDW CV is calculated mathematically as the coefficient of variation. (Dacie and Lewis, 2011).

1.2.2.2 White blood cells

The formation of white blood cells is called leukopoeisis. It occurs primarily within bone marrow and involves the following stages: pluripotintial hemopoietic stem cell, myeloblast, promyelocyte, eosinophil, neutrophil, basophilic myelocyte, band cell and granulocytes, it can be stimulated by Candida albicans .Granulocytes production is stimulated by Granulocyte-colony stimulating factor (G-CSF), also known as colony stimulating factor 3(CSF3) (Deotare *et al.*, 2015).

White blood cells are part of the body's immune system, they destroy and remove old or aberrant cells and cellular debris, as well as attack infectious agents (pathogens) and foreign substances. Normal range is 4,000–11,000 leukocytes (Ganong and William 2003).

All white blood cells have nuclei which distinguishes them from other blood cells. They can be classified according to their structures (granulocytes or a granulocytes) or by cell division lineages (myeloid cells or lymphoid cells) (Lafleur, 2008).

1.2.2.2.1 Granulocytes

Neutrophils:

They are the most abundant white blood cell, conistituting 60-70% of the circulating leukocytes (Bruce *et al.*, 2002)

They defend against bacterial or fungal infection. They are usually first responders to microbial infection; their activity and death in large numbers form pus. They are commonly referred to as polymorph nuclear (PMN) leukocytes, although, in the technical sense, PMN refers to all granulocytes. (Saladin and Kenneth 2012*).*

Neutrophils are the most common cell type seen in the early stages of acute inflammation. The life span of a circulating human neutrophil is about 5.4 days (Pillay *et al.*, 2010).

Eosinophils:

Eosinophils compose about 2-4% of the WBC total. This count fluctuates throughout the day, seasonally, and during menstruation. It rises in response to allergies, parasitic infections, collagen diseases, and disease of the spleen and central nervous system. They are rare in the blood, but numerous in the mucous membranes of the respiratory, digestive, and lower urinary tracts. They primarily deal with parasitic infections. Eosinophils 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. They secrete chemicals that destroy these large parasites, such as hook worms and tapeworms, which are too big for any one WBC to phagocytize (Pillay *et al.*, 2010).

Basophiles:

Basophiles are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing the dilation of blood vessels (Falcone *et al.*, 2000).

They excrete two chemicals that aid in the body's defenses: histamine and heparin. Histamine is responsible for widening blood vessels and increasing the flow of blood to injured tissue. It also makes blood vessels more permeable so neutrophils and clotting proteins can get into connective tissue more easily. Heparin is an anticoagulant that inhibits blood clotting and promotes the movement of white blood cells into an area. Basophiles can also release chemical signals that attract eosinophils and neutrophils to an infection site (Saladin and Kenneth, 2012).

1.2.2.2.2 Agranulocytes

Lymphocyte:

Lymphocytes are much more common in the lymphatic system than in blood. Lymphocytes are distinguished by having a deeply staining nucleus that may be eccentric in location, and a relatively small amount of cytoplasm. Lymphocytes include:B cells make antibodies that can bind to pathogens, block pathogen invasion, activate the complement system, and enhance pathogen destruction and T cells include CD4+ helper T cells,CD8+ cytotoxic T cells, γδ T cells possess an alternative T cell receptor (different from the αβ TCR found on conventional CD4+ and CD8+ T cells) and natural killer cells (Abbas and Lichtman 2003).

Monocytes:

Monocytes, the largest type of WBCs, share the phagocytosis function of neutrophils, but are much longer lived as they have an extra role: they present pieces of pathogens to T cells so that the pathogens may be recognized again and killed. This causes an antibody response to be mounted. Monocytes eventually leave the bloodstream and become tissue macrophages, which remove dead cell debris as well as attack microorganisms. Neither dead cell debris nor attacking microorganisms 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. (Saladin and Kenneth 2012).

1.2.3 Thrombocytes

The formation of thrombocytes (platelets) is called thrombopoeisis. Platelets are formed in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, and are subsequently released into the vascular compartment where they play an essential role in the formation of mechanical plugs during

the normal haemostatic response to vascular injury (Baglin *et al.*, 2005), they take part in blood clotting (coagulation). Fibrin from the coagulation cascade creates a mesh over the platelet plug. Normal range 200,000–500,000 thrombocyte. (Ganong and William 2003).

1.2.3.1 Platelets indices

Mean Platelets Volume (MPV):

MPV is the average volume of individual platelets derived from the platelet histogram. It represents the mean volume of the platelet population under the fitted platelet curve multiplied by a calibration constant, and expressed in femtoliters (Dacie and Lewis, 2011).

Platelets distribution width (PDW):

It reflects the variability in the platelet size, and it is therefore increased in the presence of platelet anisocytosis (Amin *et al.*, 2004).

Platelets cirit (ptc):

It is the volume percentage that platelets match on a total volume of blood, and it is directly related to the total number of platelets (Amin *et al.*, 2004).

1.3 Smoking

1.3.1 Definition and health effect

Smoking is a practice in which a substance is burned and the resulting smoke breathed in to be tasted and absorbed into the bloodstream. Most commonly the substance is the dried leaves of the tobacco plant which have been rolled into a small square of rice paper to create a small, round cylinder called a "cigarette". In the case of cigarette smoking these substances are contained in a mixture of aerosol particles and gasses and include the pharmacologically active alkaloid nicotine; the vaporization creates heated aerosol and gas to form that allows inhalation and deep penetration into the lungs where absorption into the bloodstream of the active substances occurs.

Smoking generally has negative health effects, because smoke inhalation inherently poses challenges to various physiologic processes such as respiration. Diseases related to tobacco smoking have been shown to kill approximately half of long-term smokers when compared to average mortality rates faced by non-smokers. Smoking caused over five million deaths a year from 1990 to 2015 (Reitsma *et al.,* 2017).

Tobacco smoke is a complex mixture of over 5,000 identified chemicals, of which 98 are known to have specific toxicological properties. The most important chemicals causing cancer are those that produce DNA damage since such damage appears to be the primary underlying cause of cancer (Talhout *et a.l,* 2011).

1.3.2 The Effects of smoking on the human body

It is widely known that smokers have higher risk for cardiovascular diseases, hypertension, inflammation, stroke, clotting disorder, and respiratory disease (Abel *et al.*, 2005). Moreover, cigarette smoking accelerates pathogenesis in different type of cancers such as lung, pancreas, breast, liver and kidney (Islam *et al.*, 2007).

Similarly, it also enhances pH in stomach that causing peptic ulcers and gastric diseases (Kume *et al.*, 2009). During past decade, it was suggested that cigarette smoking affect the blood characteristics and leads to death. Male and female smokers lose an average of 13.2 and 14.5 years of their life, respectively. At least half of all lifelong smokers die earlier as a result of smoking. The risk of dying from lung cancer before age 85 is 22.1% for a male smoker and 11.9% for a female current smoker, in the absence of competing causes of death. The corresponding estimates for lifelong nonsmokers are a 1.1% probability of dying from lung cancer before age 85 for a man of European descent, and a 0.8% probability for a woman. Smoking

one cigarette a day results in a risk of heart disease that is halfway between that of a smoker and a non-smoker (Kumar and Abul 2007).

1.3.3 Effect of cigarette smoking on blood cell

1.3.3.1 Red blood cells

Because red blood cell (RBC) membrane lipids are rich in poly-unsaturated fatty acids; therefore, the oxidative effects of oxygen on RBC membranes are greater than on other tissues. RBCs contain hemoglobin, which is one of the most potent catalysts of lipid peroxidation. The invasion of the RBC membrane by peroxidants, which occurs with hemoglobinopathies, radioactive radiation, the consumption of oxidative drugs, increased levels of certain metals and the decreased function of antioxidant systems, can lead to RBC hemolysis. In addition to causing lipid peroxidation. Peroxidants can cause the oxidation of -SH groups in proteins and RBC membranes. The -SH groups are highly reactive and can be a target during oxidative stress. Glutathione directly protects membrane proteins and preserves their stability. Decreased levels of glutathione lead to a decrease in -SH groups (Poli, 1993).

1.3.3.2 HB

Cigarette smoke has 4000substances among which CO and tars are the main toxic substances. CO can diffused rapidly across the alveolar capillaries, bind firmly to HB (with binding ability of 200-250 times greater than that of O2) forming HBCO and is a leading cause of tissue hypoxia leading to increased values of HB.

Carboxyhemoglobin also shifts the HB dissociation curve to the left side, resulting in a reduction in the ability of HB to deliver oxygen to the tissue (Blumenthal, 2001).

1.3.3.3 PCV

Smoking a major cause of polycythaemia in smokers, occupation by carbon monoxide of binding sites on hemoglobin reduces the oxygen content of circulating blood. Carbon Monoxide also increases the affinity for oxygen of the remaining haem sites; the haemoglobin-oxygen dissociation curve is left-shifted1, and there are also changes in the shape of the curve2. This result in a reduction of oxygen delivery to the renal oxygen sensor responsible for erythropoietin release which may result in polycyth-aemia. Although A reduced plasma volume is found in many smokers with a raised haematocrit,it is not a universal finding(Wickramasinghe and Weatherall, 1982).

1.3.3.4 WBCs

White blood cell count may also be a marker of exposure to toxic substances. Nicotine which is a component of cigarette smoke, stimulates cathecolamine release, and induces an increase in cortisol levels. Increases in peripheral blood WBC count, and alterations in WBC function can be the result of direct damage stemming from alterations in epithelial, and endothelial surfaces and/or cytokine levels (especially IL-6) caused by components of cigarette smoke (Smith *et al*.,2003).

1.3.3.5 Platelets

Concerning platelet disturbance in smoking, conflicting results have emerged from the studies of platelet aggregation showing that smoking causes either an increase or a decrease in the *in vitro* aggregation response of platelets after the addition of ADP in smokers as compared with non-smokers, while other studies have reported no difference (Hioki *et al.*, 2001).

1.3.4 Smoking in Sudan

In Sudan there is no protection from smoking that means there is no Smoke free (health care facilities, universities, government facilities indoor offices, restaurants and public transport) in all other indoor public places,

smoke free are not available and there are no funds for smoke free nforcement.

WHO (2011 and 2012) is best practice of warn about the dangers to whole population in a media campaign because it ran a national anti-tobacco campaign, it was a part of a comprehensive tobacco control program, it was pre-tested with the target audience, were conducted a target audience research. Aired on television and/or radio, utilized media planning earned Media/Public relations were used to promote the campaign, process evaluation was used to assess implementation and, outcome evaluation was used to assess effectiveness. All that things are not available in Sudan.

- **Warn about the dangers to tobacco users on product packaging:**

In Sudan the warning label is by text, has 15% of pack cover, there is graphic warning round and there is no standardize packaging. In this part Australia is the best practice.

WHO (2012) benchmark is 75% of retail price is excise tax. In Sudan 58% of retail price is excise tax.

1.4 Rationale

Tobacco use leads most commonly to diseases affecting the heart and lungs, with smoking being a major risk factor for heart attacks, strokes, chronic obstructive pulmonary disease (COPD), emphysema, and cancer (particularly lung cancer, cancer of the larynx and mouth, and pancreatic cancer). Cigarette smoking increases the risk of Crohn's disease as well as the severity of the course of the disease it is also the number one cause of bladder cancer. Smoking is the greatest avoidable cause of disease and death. Every year more than 5,050 people in Sudan are killed by tobacco-caused disease, while more than 142,200 children and more than 1 million adults continue to use tobacco. During the last 20 years the amount of tar and Nicotine content delivered by cigarette made by United States has decreased more than 50%.WHO (2011) estimates that by the decade 2020-2030 tobacco will be responsible for 10 million deaths per year, with 70% occurring in developing countries (Nancy and Derek, 2000).

About 1.3 billion people are regular smokers worldwide and every day between 8,200 and 9,900 young people start to smoke, risking rapid addiction to nicotine this work (Shafquat *et al.*, 2007).

1.5 Objectives

1.5.1 General Objective

To evaluate complete heamogram in Sudanese cigarette smokers.

1.5.2 Specific Objectives

To measure RBCs and it indices, HB, PCV, MCV, MCH, MCHC, RDWSD ,RDWCV,TWBCs and differentials, platelets and its indices.

To measure CBC in smokers and non-smokers

To study effect of smokers' ages, number of cigarettes consumed per day, the duration of smoking on complete blood counts.

Chapter Two

Materials and Methods

Chapter Two

Materials and Methods

2.1 Study design

It is an analytical case control study.

2.2 Study area

This study was carried out during the period from September 2015 to May 2016 in Khartoum North.

2.3 Study population

Ninety adult male cigarette smokers and seventy adult male nonsmokers were enrolled in the study.

2.4 Inclusion criteria

Adult healthy male cigarette smokers at different ages, consumed ≥ 5 cigarettes per day and smoke for≥ 2 years

2.5 Exclusion criteria

Smokers with history of diseases that may affect the results like: Diabetes, hypertension, anemia, thrombosis .

2.6 Data collection

Data was collected using self administered questionnaire designed to obtain demographic data, include name, age, number of cigarette per day (5 and more), duration of smoking per year (more than 2 years), history disease hypertension, anemia, and diabetes, educational level and marital status.

2.7 Specimen collection

Blood of 2.5 ml were collected using sterile disposable syringe and drawn into EDTA containers.

2.8 Laboratory investigation

Complete blood count was investigated using hematological analyzer Sysmex KX-21N -Japan.

2.8.1 Quality Control of automated Haematology Sysmex Kx-21:

This program is used to set QC method and output method of QC data.They are two kinds: X control (control blood is subjected to two consecutive analysis and the mean of them is used as the QC data. This method causes little influence on reproducibility in analysis).The other is L-J control (this control uses data from a single analysis of control blood as QC data.the control width in this method is prone to influence in reproducibility in analysis ,so that the control width is wider than in the first method) (Sysmex corporation ,1998)**.**

2.8.2 Analysis parameters

This instrument analysis the following parameter using three detectors block and two kind of reagent:

WBCs analysis principle: DC detection method WBCs count in 1ml of Whole blood:

- LYM% (W-SRC) (WBC-small cell ratio), ratio % of small lymphocyte to whole blood.
- LYM# (W-SCC) (WBC-small cell count).
- Absolute count of small lymphocyte in one micro liter of whole blood.
- NEUT# (W-LCC) (WBC-large cell count).
- Absolute count of the neutrophils in one micro liter of whole blood.

RBCs (red blood corpuscular) analysis principle DC detection method:

- RBC count in 1ml of whole blood.
- HBG (hemoglobin) analysis principle nonocyanide hemoglobin analysis method volume (gram) of hemoglobin in 1dl of whole blood.

2.9 Reagents

Instrument reagent system is composed of 5 reagents for testing and cleaning:

- Cell Pack ---------- dilute sample.
- Cell wash ---------- RBC dilution.
- WDTM lyses ---------- WBC dilution.
- Stromatolyser CTM ----- hemoglobin dilution.
- 20% Clorox ---------- cleaning.

2.10 Statistical Analysis

The characteristics of study groups (number of consumed cigarettes per day, ages of smokers and the duration of smoking) were presented by frequency.

Effect of smoking on CBC was determined by student Independent T test.

One-way ANOVA was used to show the effect of age, duration of smoking and number of cigarette on complete blood count of smokers.

Statistical package for social science (SPSS version 11.5) program was used for all these above mentioned tests.

Significance level was set as $P \leq 0.05$.

2.11 Ethical consideration

The study was approved by the Medical Laboratory College Committee −SUST.Awritten consent was obtained from the participants after they had been informed with the objectives, benefits and expected outcomes of the study. The participants were assured that the collected information will be kept confidential and will not be used for any other purpose other than this study.

Chapter Three

Results

Chapter Three

Results

3.1 Characteristics of the study population

The distribution of the smokers according to their age:

The highest frequency (55) was recorded by the age group (20 – 35) years old and the lowest frequency (2) by the group of >50 years. **Table (1)**

The distribution of the smokers according to the number of consumed cigarette per day:

The groups of smokers consuming (5 – 10) cigarettes per day recorded the highest frequency (56) and the group (16–20) cigarettes per day recorded the lowest frequency (15) of smoking. **Table (2)**

The distribution of smokers according the duration of smoking

The duration of smoking (8 – 15) years recorded the highest frequency (40) and the group smoke (16–21) years recorded the lowest frequency (8). **Table (3)**

3.2 Effect of smoking on complete blood counts

Smoking caused significant increase ($P \leq 0.05$) in RBCS, HB, HCT, MCV, MCH, RDWSD and RDWCV. **Table (4)**

Table (5) shows the effect of smoking on TWBCs and differential count. Significant increase was found ($P \leq 0.05$) in neutrophils, monocytes and eosinophils.

Table (6) shows the effect of smoking on platelets count and indices. A significant decrease was observed in platelets count ($P \geq 0.05$).

3.3 Effect of consumed cigarette per day on complete blood counts:

Number of cigarette consumed per day did not affect the RBCs and its indices except of the group consumed (16 – 20) cigarettes per day recorded a significant (P≤0.05) higher values of RDWCV than the others two groups. **Table (7)**

Number of cigarette consumed per day did not affect the TWBCs or differential count **Table (8)** also it did not affect platelets count and indices **Table (9)**.

3.4 Effect of the smokers' age on complete blood counts:

Variations on the values of HB, TWBCs and neutrophils were found among the different age groups. **Tables (10 and 11).**

Platelet count and indices were not affected. **Table (12)**

3.5 Effect of the duration of smoking on complete blood counts:

The group with a duration of smoking (2–7) years showed a significant (P≥0.05) decrease in HB and HCT. **Table (13)**

Neither TWBCs and differential count nor platelet count and indices were affected by the duration of smoking **Tables (14 and 15).**

Characteristic of study groups

Table 1: Distribution of smokers according to their ages

Group/years	Frequency	Percent
less than 20	**10**	**6.3**
20----35	**55**	**34.4**
36-----50	**23**	**14.4**
more than 50	**2**	**1.3**

Table 2: Distribution of smokers according to the number of cigarettes consumed per day

Number of cigarette consumed /day	Frequency	Percent
5----10	**56**	**35.0**
11-----15	**19**	**11.9**
16-----20	**15**	**9.4**

Table 3: Distribution of smokers according to their duration of smoking in years

Duration of smokig/years	Frequency	Percent
2----7	**40**	**25.0**
8-----15	**42**	**26.3**
16-----21	**8**	**5.0**

Table 4*:* Effect of smoking on erythrocytes parameters

Parameter	Non-smokers M±SD	Smokers M±SD	P value
RBC×10^{12} /l	4.91±0.58	5.16±0.61	0.01
HB g/dl	13.92±1.23	15.03±1.21	0.00
HCT %	41.63±5.96	46.42±8.34	0.00
MCV fl	84.79±5.76	88.44±6.51	0.00
MCH pg	27.99±2.24	29.16±2.78	0.01
MCHC g/dl	32.86±1.69	32.97±1.21	0.64
RDWSD fl	43.95±3.89	46.98±5.21	0.00
RDWCV %	13.78±1.18	14.25±1.30	0.02

Significance level($P \leq 0.05$)

Table 5*:* Effect of smoking on total white blood cells and differential counts

Parameter	Non-smokers M±SD	Smokers M±SD	P value
TWBCs×10^9 /l	6.03±2.05	7.16±2.41	0.00
Neutrophil/ µl	3.27±1.60	4.28±2.11	0.00
Lymphocyte/ µl	2.17±0.73	2.11±0.64	0.58
Monocyte / µl	0.41±0.19	0.62±0.22	0.00
Eosinophil/ µl	0.21±0.13	0.26±0.02	0.56
Basophil/ µl	00	00	---

Significance level($P \leq 0.05$)

Table 6*:* Effect of smoking on platelets and indices

Parameter	**Non-smokers M±SD**	**Smokers M±SD**	**P value**
Platelet$\times 10^9$ /l	238.23±36.89	212.30±55.71	0.05
PDW fl	12.60±2.10	12.30±1.92	0.34
MPV fl	9.85±0.93	10.83±2.51	0.39
P-LCR %	24.68±7.11	24.10±6.85	0.60

Significance level($P \leq 0.05$)

Table 7*:* Effect of number of cigarettes consumed on red cell's indices

Parameter	Number of cigarette consumed /day	M±SD
RBCS×10^{12} /l	5 –10	5.18±0.65
	11 –15	5.28±0.37
	16 – 20	5.15±0.79
HB g/dl	5 –10	15.09±1.21
	11– 15	14.96±1.19
	16 – 20	14.77±1.18
HCT%	5 – 10	45.88±3.42
	11– 15	45.37±3.32
	16 – 20	44.35±4.32
MCV fl	5 – 10	87.71±4.89
	11 – 15	87.23±4.81
	16 – 20	88.29±6.48
MCH pg	5 – 10	29.29±2.91
	11 – 15	28.13±2.02
	16 – 20	29.26±3.17
MCHC%	5 – 10	32.93±1.22
	11 – 15	32.94±0.82
	16 – 20	33.17±1.45
RDWSD fl	5 – 10	46.46±5.21
	11 – 15	46.51±4.39
	16 – 20	48.93±6.32
RDWCV %	5 – 10	14.01±0.92^{b}
	11 – 15	14.36±1.51^{ab}
	16 – 20	15.00±1.91^{a}

Significance level($P \leq 0.05$)

Table8*:* Effect of number of cigarettes consumed on total white blood cell and differential counts

Parameter	**Number of cigarette consumed/ day**	**M±SD**
TWBCs×10^9 /l	5 – 10	6.73±1.63
	11 – 15	7.11±2.08
	16 – 20	7.86±3.41
Neutrophils/ µl	5 – 10	4.32±2.08
	11 – 15	4.81±2.03
	16 – 20	4.77±2.16
Lymphocytes/ µl	5 – 10	2.13±0.67
	11 – 15	2.06±0.64
	16 – 20	2.12±0.58
Monocytes/ µl	5 – 10	0.66±0.24
	11 – 15	0.60±0.20
	16 – 20	0.64±0.22
Eosinophils/ µl	5 – 10	0.29±0.07
	11 – 15	0.20±0.09
	16 – 20	0.21±0.06
Basophils/ µl	5 – 10	0.00±0.00
	11 – 15	0.00±0.00
	16 – 20	0.00±0.00

Significance level($P \leq 0.05$)

Table9: Effect of number of cigarette consumed on platelets and indices

Parameter	Number of cigarette consumed/ day	M±SD
Platelets×10^9 /l	5 – 10	215.42±81.07
	11 – 15	210.22±71.62
	16 – 20	201.00±57.33
PDW fl	5 – 10	12.33±1.88
	11 – 15	12.78±2.10
	16 – 20	11.44±1.69
MPV fl	5 – 10	9.86±0.81
	11 – 15	9.86±0.89
	16 – 20	9.68±1.19
PLCR %	5 – 10	24.46±6.66
	11 – 15	24.57±7.82
	16 – 20	22.81±8.00

Significance level($P \leq 0.05$)

Table10: Effect of smoker's age on Red blood cell series

Parameter	Age (years)	Mean± SD
RBCs×10^{12} /l	Less than 20	5.29±0.43
	20 – 35	5.14±0.70
	36 – 50	5.22±0.40
	More than 50	4.65±0.35
HB g/dl	Less than 20	15.10±1.20[a]
	20 – 35	15.03±1.31[a]
	36 – 50	15.15±0.89[a]
	More than 50	13.15±0.21[b]
HCT%	Less than 20	46.31±3.14
	20 – 35	45.40±4.13
	36 – 50	45.77±2.56
	More than 50	45.40±5.80
MCV fl	Less than 20	89.59± 6.86
	20 – 35	88.41± 7.19
	36 – 50	87.79 ±4.48
	More than 50	89.40± 7.92
MCH pg	Less than 20	29.47±3.00
	20 – 35	29.23±3.08
	36 – 50	28.83±1.87
	More than 50	28.95±2.76
MCHC%	Less than 20	32.82±1.28
	20 – 35	32.99±1.27
	36 – 50	33.05±1.12
	More than 50	32.35±0.21

RDWSD fl	Less than 20	47.12±3.73
	20 – 35	47.39±5.66
	36 – 50	45.77±4.84
	More than 50	48.35±5.30
RDWCV %	Less than 20	14.23±0.87
	20 – 35	14.36±1.36
	36 – 50	14.01±1.40
	More than 50	14.20±0.28

Significance level($P \leq 0.05$)

Table11: Effect of smoker's age on white blood cell and differential count

Parameter	Age group	Mean± SD
TWBC $\times 10^9$ /l	Less than 20	8.64±3.07[a]
	20 – 35	6.92±2.07[b]
	36 – 50	6.70±1.73[b]
	More than 50	6.15±2.33[b]
Neutrophils/ µl	Less than 20	5.17±1.86[a]
	20 – 35	4.03±1.68[b]
	36 – 50	3.83±1.35[b]
	More than 50	2.90±0.78[ab]
Lymphocytes/ µl	Less than 20	2.51±0.80
	20 – 35	2.02±0.60
	36 – 50	2.17±0.59
	More than 50	1.85±0.64
Monocytes/ µl	Less than 20	0.71±0.26
	20 – 35	0.66±0.27
	36 – 50	0.50±0.22
	More than 50	0.50±0.00
Eosinophils/ µl	Less than 20	0.18±0.07
	20 – 35	0.30±0.19
	36 – 50	0.20±0.12
	More than 50	0.25±0.07
Basophils/ µl	Less than 20	0.00±0.00
	20 – 35	0.00±0.00
	36 – 50	0.00±0.00
	More than 50	0.00±0.00

Significance level($P \leq 0.05$)

Table 12: Effect of smokers' age on platelets count and its indices

Parameter	Age group	Mean± SD
Platelets×10^9 /l	Less than 20	243.92±79.00
	20 – 35	212.70±68.78
	36 – 50	195.95±56.10
	More than 50	191.50±0.71
PDW fl	Less than 20	12.33±1.59
	20 – 35	12.45±2.13
	36 – 50	11.82±1.57
	More than 50	12.95±1.06
MPV fl	Less than 20	9.92±0.96
	20 – 35	9.87±0.96
	36 – 50	9.65±0.71
	More than 50	10.30±0.42
PLCR %	Less than 20	24.93±7.12
	20 – 35	24.27±7.46
	36 – 50	22.91±5.34
	More than 50	27.50±2.83

Significance level($P \leq 0.05$)

Table13: Effect of the duration of smoking on Red cells series

Parameter	Duration of smoking (years)	Mean± SD
RBCs×10^{12} /l	2 – 7	5.05±0.66
	8 –15	5.39±0.53
	16 – 21	5.14±0.27
HB g/dl	2 – 7	14.91±1.35[b]
	8 – 15	15.24±0.99[a]
	16 – 21	15.10±0.94[a]
HCT%	2 – 7	44.53±3.36[b]
	8 – 15	46.12±3.91[a]
	16 – 21	47.15±2.86[ab]
MCV fl	2 – 7	87.25±4.50
	8 – 15	88.32±5.78
	16 – 21	86.73±4.43
MCH pg	2 – 7	29.63±2.94
	8 – 15	28.23±2.57
	16 – 21	29.18±1.51
MCHC %	2 – 7	33.02±1.23
	8 – 15	32.76±1.23
	16 – 21	33.40±0.97
RDWSD fl	2 – 7	46.97±6.16
	8 – 15	47.36±4.28
	16 – 21	44.99±4.62
RDWCV %	2 – 7	14.34±1.33
	8 – 15	14.27±1.32
	16 – 21	13.59±0.90

Significance level($P \leq 0.05$)

Table 14: Effect of duration of smoking on white blood cells and differential count

Parameter	Duration of smoking (years)	Mean± SD
TWBCs×10^9 /l	2 – 7	6.88±2.56
	8 –15	7.02±1.84
	16 – 21	7.45±.89
Neutrophil/ µl	2 – 7	4.54±2.43
	8 –15	4.23±1.97
	16 – 21	4.03±1.18
Lymphocyte / µl	2 – 7	2.04±0.65
	8 –15	2.24±0.62
	16 – 21	2.14±0.67
Monocytes/ µl	2 – 7	0.68±0.78
	8 –15	0.57±0.22
	16 – 21	0.44±0.12
Eosinophils/ µl	2 – 7	0.29±0.80
	8 –15	0.23±0.10
	16 – 21	0.15±0.05
Basophiles/ µl	2 – 7	0.00±0.00
	8 –15	0.00±0.00
	16 – 21	0.00±0.00

Significance level($P \leq 0.05$)

Table15: Effect of duration of smoking per years on platelets and platelets indices

Parameter	Duration of smoking per years	Mean± SD
Platelets×10^9 /l	2 – 7	219.13±86.91
	8 –15	203.32±55.15
	16 – 21	197.63±55.11
PDW fl	2 – 7	12.45±2.09
	8 –15	12.04±1.62
	16 – 21	12.11±1.79
MPV fl	2 – 7	9.97±0.96
	8 –15	9.71±0.79
	16 – 21	9.78±0.92
PLCR %	2 – 7	25.50±6.66
	8 –15	23.08±6.38
	16 – 21	24.14±6.09

Significance level($P \leq 0.05$)

Chapter Four

Discussion

Discussion:

The study aimed to determine the effect of smoking on complete blood count.

Smoking showed significant increase in the RBCs which accords with the finding of (Tarazi *et al.*, 2008 and Kume *et al.*, 2009). This can lead to polycythemia which slow blood velocity and increase risk of intravascular clotting.

HB concentration showed significant increase in smokers which is on lined with the study of (Iqbal *et al.*, 2003 and Mahsud *et al.*, 2010).

HCT showed significant increase due to smoking this agrees with the previous studies of (Tarazi *et al.*, 2008, Kume et al, 2009 , Hassan *et al.*, 2012 and Jayballabh *et al.*, 2013). Elevated levels of hematocrit lead to polycythemia vera (PV), a myeloproliferative disorder in which the RBCs are produced excessively by bone marrow, and also related to an increased risk of developing atherosclerosis and cardiovascular diseases (Ferro *et al.*, 2004).

MCV, MCH and MCHC are three main red blood cell indices that help in measuring the average size and hemoglobin composition of the red blood cells. There was significant increase in MCV, MCH RDWSD and RDWCV were significantly increased, this may indicate the presence of anisopoikilocytois (Ghosh *et al.*, 2012).

TWBCs showed significant increase in smokers this is on line with study of Kawada, (2004). Previous study reported that high level RBC, WBC and Haematocrit are associated with blood viscosity and clotting as result of smoking (HoCH, 2004).

The reduction on platelets count mean value found in this work agrees with Compbell *et al.*, (2008). Mean platelet volume (MPV) or platelet distribution width (PDW) or platelet large cell ratio PLCR did not alter

with smoking, this accords with the study of (Butkiewicz *et al.*, 2006) And (Arsian *et al.*, 2008).

Age of smokers' increase of HB, TWBCs, and neutrophils. Number of cigarette consumed per day increase of RDWCV. Duration of smoking elevate HB and HCT.

Conclusion:

Smoking caused:

Significant increase in erythrocytes count, HB level, PCV, MCV, MCH, TWBCs (neutrophil, monocytes) and significant decrease in platelet count. The age of smokers increase HB concentration, TWBCs,and absolute neutrophils counts.

Number of cigarette consumed per day increase RDWCV. The duration of smoking elevate HB concentration and HCT.

Recommendations:

Further studies are needed with large sample size and to cover other States in Sudan.

Raise the people awareness of tobacco smoking through media campaign and general education.

Protect from passive smoking, that any indoor public places must be smoke free.

Raise taxes as a percent of cigarette price.

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Appendix

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire to measure CBC in Sudanese cigarette smokers

Age (…)

Education level

Illetrate (…) Primary (…) Secondary (…) University (…)

Marital status

Married (…) Not married (…)

Did you smoke cigarette

Yes (...) No (…)

Number of cigarette per day (…..) (Five and above)

Duration of smoking (……) (two years and above)

Suffer from diseases: Hypertension (...) Diabetes (...) Anemia (...) thrombosis (…)

Result: WBC……..…RBC…...……HGB ……HCT……..…….

MCV……MCH……MCHC…… PLT……..….

LYM%……NEUT%……LYM#……NEUT#……

RDWcv……RDWsd……PDW……MPV……

PLCR……

Signature.....

Informed consent

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

ماجستير مختبرات طبية

تخصص علم امراض الدم والمناعة الدموية

قياس تعداد الدم الكامل لمدخني السجائر السودانيين في الخرطوم بحري

اقرار موافقه بالمشاركة

الاسم: .....

سوف يتم أخذ عينة من الدم (2.5 مل) من الوريد بواسطة حقنة طعن وذلك بعد مسح منطقة العينة بواسطة مطهر.

كل الأدوات المستخدمة لأخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية

وانا اقربان هذه العينات سوف يتم تحليلها لغرض البحث فقط.

أوافق أنا المذكور اعلاه أخذ عينة لإجراء الدراسة

الإمضاء.....

التاريخ: .....