Sudan University of Science and Technology
College of Graduate Studies

Estimation of Complete Blood Count (CBC) in Toombak dippers Khartoum State, 2019

قياس تعداد الدم الكامل عند متعاطي التمباك في ولاية الخرطوم، 2019

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

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الآيـة

بسم الله الرحمن الرحيم

قال تعالى:

إن الله وملائكته يصلّون على النبي يا أيها الذين آمنوا صلوا عليه وسلموا تسليما (56)

صدق الله العظيم
سورة الاحزاب

Dedication

To my Family for their love,,,
To my husband for his motivation,,,
To my Friends for their support,,,
And to my colleagues…
I dedicate this work with my
Best wishes to all.

Acknowledgement

I would firstly like to thank Allah for giving me knowledge, patience and support to complete this work. I am deeply indebted to my supervisor Prof: Shadia Abdlatii Omer
For her guidance help and support of revising the text and giving valuable advice throughout this work and had never preserved her help me.

Abstract:

This is an analytical case control study conducted at Khartoum State during the period from September 2018 to April 2019 to investigate the effects of toombak dipping on the complete blood cell count (CBC) of the dippers. Thirty fife adult healthy toombak dippers and fifty healthy non toombak dippers were enrolled in the study after a written consent had been obtained from them. The ethical approval also was obtained from the College of Medical Laboratory Sciences-SUST. The socio demographic data of the participants were collected by a questionnaire.

Venous blood (2.5 ml) was collected in EDTA containers and CBC was determined using an automated hematological analyzer (Hemolyzer pro 3). The obtained data were analyzed by the using the software program statistical package of social science (SPSS.V.25).

Toombak dippers showed significantly (P>.05) higher values than the control group in the mean values of: total leucocytes count ($x10^9$/l) (6.4 ±1.83 vs. 5.7 ±92), erythrocytes count ($x10^9$/l) (5.5 ±.83 vs. 5.1 ±.41), hematocrit (%) (45.9 ±6.32 vs. 43.2 ±3.69), red cell distribution width (%) (16.0 ±1.23 vs. 13.6 ±1.24), and platelet count ($x10^9$/l) (290.4±100.96 vs. 246.4 ±53.270); and significantly (P>.05) lower values than the control group in the mean values of: mean cell hemoglobin (pg) (27.4 ±3.56 vs. 28.7 ±1.89), mean corpuscular hemoglobin concentration (g/dl) (32.5 ±3.17 vs. 34.0 ±.96) and mean platelet volume (fl) (8.6 ±.98 vs. 10.2 ±1.14).

The younger toombak dippers showed significantly higher values in WBC (LYM ($x10^9$/l)) than the older ones, the duration of dipping, numbers of dipping / day and the site of dipping has no effect on CBC results.

The conclusion is that toombak dipping affects on CBC.

Key word: *CBC, Toombak, dipping.*

المستخلص:

هذه دراسة تحليلية محكِّمة أجريت في ولاية الخرطوم خلال الفترة من سبتمبر 2018 إلى أبريل 2019 للبحث في تأثير تعاطي التمباك على العد الكلي للدم عند مستخدميه ، سُجل خمسة وثلاثين متعاطياً للتمباك بصحة جيدة وخمسين غير متعاطين للتمباك في الدراسة بعد الحصول على موافقة كتابية منهم، كما تم الحصول على الموافقة الأخلاقية من كلية علوم المختبرات الطبية (جامعة السودان للعلوم والتكنلوجيا). جُمعت البيانات الاجتماعية السكانية من المشاركين عن طريق الإستبيان.

جُمعت 2.5 مل من الدم الوريدي في حاويات EDTA وحُدد العد الكلي للدم باستخدام محلل الدم الالي (3Hemolyzer pro). حُللت البيانات التي تم الحصول عليها بواسطة (T.test\ ANOVA) باستخدام برنامج الحزمة الإحصائية للعلوم الاجتماعية (V. SPSS. 25).

أظهر متعاطو التمباك قيماً مرتفعة ذات دلالة إحصائية معنوية(<.05) بالمقارنة مع الفئة المحكِّمة في: إجمالي عدد الكريات البيضاء(10^9/ ل) (6.4 ± 1.83 مقابل 5.7 ± 92) ، عدد كريات الدم الحمراء(10^9/ ل) (5.5 ± .83 مقابل 5.1 ± .41) ، حجم الكريات المتراصة (%) (45.9 ± 6.32 مقابل 43.2 ± 3.69) ، عرض توزيع الخلية الحمراء(%) (16.0 ± 1.23 مقابل 13.6 ± 1.24) ، وعدد الصفائح الدموية (10^9/ ل) (290.4 ± 100.96 مقابل 246.4 ± 53.27), وقيماً منخفضة ذات دلالة إحصائية معنوية (<.05) بالمقارنة مع الفئة المحكِّمة في : متوسط خضاب الدم في الخلايا (بيكو جم) (27.4 ± 3.56 مقابل 28.7 ± 1.89) ، متوسط تركيز خضاب الدم الحبيبي(جم / ديسل) (32.5 ± 3.17 مقابل 34.0 ± .96) و متوسط حجم الصفائح الدموية (فيمتو ل) (8.6 ± .98 مقابل 10.2 ± 1.14).

أظهر متعاطو التمباك الأصغر سناً قيما مرتفعة ذات دلالة إحصائية معنوية في الدم الأبيض (الخلايا الليمفاوية (10^9/ ل)) بالمقارنة مع متعاطي التمباك الأكبر. مدة تعاطي التمباك, عدد مرات التعاطي في اليوم ومكان وضع التمباك في الفم لم يؤثر على العد الكلي للدم.
من هذه الدراسة خلصنا إلى أن تعاطي التمباك يؤثر على العد الكلي للدم.

***مفاتيح الكلمات** : العد الكلي للدم, التمباك, التعاطي.*

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Abbreviations:

AGM	Aorta-gonads-mesonephros
CBC	Complete blood count
CFU	Colony forming units
CFUE	Colony forming unit erythroid
EDTA	Ethylene Di-amine Tetra-acetic Acid
FBC	Full blood count
FBE	Full blood exam
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte-macrophage colony stimulating factor
Tpo	Thrombopoietin
IL	Interleukin
HB	Hemoglobin
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MCHC	Mean corpuscular hemoglobin concentration
HCT	Hematocrit
HDW	Hemoglobin distribution width
RDW	Red cell distribution width
PCV	Packed cell volume
PMNs	Polymorph neutrophils
NNN	Nnitrosonornicotine
SLT	Smokeless tobacco
TSNAs	Tobacco specific N-nitrosamine
NNK	Nicotine-derived nitrosamine ketone

CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

Introduction:

The tobacco epidemic is a major public health problem and one of the main causes of death and disability worldwide (WHO, 2017). More than 300 million people in at least 70 countries, use smokeless tobacco (SLT) (NCI, 2014). SLT is used without burning the product, and can be used orally or nasally. Internationally, there are more than twenty eight types of orally used and two types of nasally inhaled ST (Kuper, *et.al*., 2002; Rodu and Jansson, 2004). Oral ST Products are positioned in the mouth, cheek, or lip, and are sucked (dipped) or chewed. Chewing tobacco can be classified as loose leaf (made from cigar leaf tobacco that is air-cured, sweetened, and loosely packed), plug (made from heavier grades of tobacco leaves harvested from the top of the plant, immersed in a mixture of licorice and sugar and pressed into a plug), or twist (air cured or fire-cured burley tobacco leaves, flavored and twisted in form of a rope) (Hoffmann and Hecht, 1985). Snuff is a universal word for finely cut or powdered, flavored tobacco, which can be prepared as moist snuff (air-cured and fire-cured tobacco, flavored and powdered into fine particles, containing 20-55% moisture by weight) and dry snuff (fire-cured, fermented tobacco powder that may contain aroma and flavor additives) (Boffetta, *et.al*., 2008). Tobacco pastes or powders are also used orally and applied to the gums or teeth. Dry snuff can also be inhaled through the nasal passages (Boffetta, *et al*., 2008). In the United States, the major types of ST are chewing tobacco (cut tobacco leaves) and snuff (moist ground tobacco). In the Sudan, snuff€ , locally known as toombak, was introduced approximately 400 years ago. It is always processed into a loose moist form, and its use is

widespread in the country. Tobacco used for manufacture of toombak is of the species *Nicotiana rustica*, and the fermented ground powder is mixed with an aqueous solution of sodium bicarbonate. The resultant product is moist, with a strong aroma, highly addictive and its use is widespread particularly among males (Idris, *et.al*., 1998a). Table 1 shows an example of four types of ST in the four different countries.

Use of SLT products can have a significant impact on oral health and can contribute to the development of oral mucosal conditions. These manifestations can range from mild, localized reversible inflammations likes snus induced lesions, to more severe forms such as oral leukoplakia and oral cancer for example nass/naswar, shammah, toombak, betel nut (Keith, *et .al*, 2017). A summary of SLT products, the countries they are commonly used in and the associated potential health effects is presented in table 1.2.

Table1.1: Some types of smokeless tobacco (Alsanosy, 2014)

Country	**Local name(s)**	**Method of use**	**Method of preparation**	**References**
Sudan	Toombak	Rolled into a ball that weighs about 10 g and is called a saffa. The saffa is held between the gum and the lip or cheek, or under the tongue on the floor of the	Of the species *Nicotiana rustica*, and the fermented ground powder is mixed with an aqueous solution of sodium bicarbonate. The resultant product	(Idris, *et.al.*, 1998a)

		mouth.	is moist, with a strong aroma	
Saudi Arabia	Shammah	Placed in the mouth as a quid.	mixture of powdered tobacco, carbonate of lime, ash, black pepper, oils and flavoring	(Allard, *et.al*., 1999)
Sweden	Snuff, (locally known also as snus)	Snus is manufactured into a dry form used in the nasal cavity and a moist form used in the oral cavity.	Finely ground (powdered) tobacco that is sold moist, dry, or in tea bag-like pouches called sachets.	(Idris, *et.al*., 1998a)
Turkey	Maras powder	Applied to the mucosa of the lower lip for 4-5 min and then it is spit out.	The leaves of the plant (Nicotianarustica) are powdered mixed with the ash.	(Özkul, *et.al*., 1997)

Table 1.2: Consumption of SLT in the South East Asia region. (Keith, *et.al*, 2017)

Product	**Regions**	**Potential Effects**
Snuff	Africa, Latin America	Oral Cancer (Ayo-Yusuf, *et .al*, 2000),Oral Keratotic Lesions (Hille, *et.al*,1996), Gingival Recession, Leukoplakia, And Tooth Loss (Agbor, *et.al*, 2013)
Swedish Snus	Europe	Localized Inflammation, Snuff Induced Keratotic Lesion (Roosaar, *et .al*,2006)
Nasway/Naas /Naswar	Europe,Middle East	Oral Premalignant Lesions, Oral Leukoplakia ,Chronic Esophagitis (Evstifeeva and Zaridze ,1992),Oral Squamous Cell Carcinoma (Merchant, *et .al*, 2000)
Khat /Q'at	Middle East	Loss Of Periodontal Attachment (Hill and Gibson,1987), Xerostomia ,Tooth Staining, White Mucosal Lesion, Psychological And Sympathomimetic Effects (Yarom, 2010) Digestive And Genitourinary Symptoms (Warfa, *et.al* 2007)
Shammah	Middle East	Oral Leukoplakia ,Oral Cancer (Al-Tayar, *et.al* ,2015)
Betel Quid2 Paan/Paan Masala	Asia	Addictive Properties, Oral Sub mucosa Fibrosis (Precancerous) (Nair, *et.al*, 2004)
Toombak	Africa	Oral Cancer (Ahmed , 2013)
Chimó	Latin America	Increased Blood Pressure And Heart Rate, Epithelial Dysplasia, Hyperkeratosis, Acanthosis, Chronic Inflammation, Cancer (Stanfill, *et.al*, 2010)

1.1 Literature review:-

1.1.1 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 .The blood composed of plasma and form elements. 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 (Dacie and Lewis 2006).

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 likes 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 2006).

1.1.2 Haemopoiesis:

The process that leads to the production and regulation of blood cells is called hematopoiesis. It consists of mechanisms triggering differentiation and maturation of hematopoietic stem cells. Located in the bone marrow, hematopoietic stem cells are undifferentiated cells, unobservable directly

(even though they can be tracked by markers), with unique capacities of differentiation (the ability to produce cells committed to one of blood cell types) and self-renewal (the ability to produce an identical cell with the same properties). Under the action of growth factors (molecules acting like hormones playing an activator/inhibitor role), hematopoietic stem cells produce differentiated cells throughout cell divisions until blood cells (White cells, red blood cells, and platelet) are formed and ready to enter the bloodstream. Blood is a life-sustaining fluid which circulates through the heart and blood vessels. It carries oxygen and nutrients to the tissues and waste products to the lungs, liver and kidneys, where they can be removed from the body (Barbara, 2004). In the first few weeks of gestation the yolk sac is the main site of haemopoiesis. However, definitive haemopoiesis derives from a population of stem cells first observed on the dorsal aorta termed the AGM (aorta-gonads-mesonephros) region. These common precursors of endothelial and haemopoietic cells (haemangioblasts) are believed to seed the liver, spleen and bone marrow and from 6 weeks until 6-7 months of fetal life the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth . The bone marrow is the most important site from 6 to 7 months of fetal life. During normal childhood and adult life the marrow is the only source of new blood cells. The developing cells are situated outside the bone marrow sinuses and mature cells are released into the sinus spaces, the marrow microcirculation and so into the general circulation. In infancy all the bone marrow is haemopoietic but during childhood there is progressive fatty replacement of marrow throughout the long bones so that in adult life haemopoietic marrow is confined to the central skeleton and proximal ends of the femurs and humeral. Even in these haemopoietic areas, approximately 50% of the marrow consists of fat. The remaining fatty marrow

is capable of reversion to haemopoiesis and in many diseases there is also expansion of haemopoiesis down the long bones. Moreover, the liver and spleen can resume their fetal haemopoietic role ('extra modularly haemopoiesis) (Hoffbrand, *et.al,* 2006).

1.1.3 Formed elements of the blood:

1.1.3.1 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 normoblast, 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).

1.1.3.1.1 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 hem group. The molecular weight of HB is 68000 Dalton. Each Red cell contains approximately 640 million hemoglobin molecules (Hoffbrand *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 hem 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, 2006).

1.1.3.1.2 Hematocrit:

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

1.1.3.1.3 Red cell indices:

Mean cell volume (MCV):

MCV is measured directly, but in semi-automated counters MCV is calculated by dividing the PCV by RBC (femtoliters).The MCV has been used to guide the diagnostic workup in patients with anemia, for example testing patients with microcytic anemia for iron deficiency or thalassemia, 16 and those with macrocytic anemia for folate or vitamin B_{12} deficiency (Griner and Oranburg, 1978).

Mean cell hemoglobin (MCH):

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

Mean cell hemoglobin concentration (MCHC):

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

1.1.3.1.4 Red cell distribution width (RDW):

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

1.1.3.2 White blood cells:

The formation of white blood cells is called leukopoiesis. 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).

1.1.3.2.1 Granulocytes:

Neutrophils: They are the most abundant white blood cell, constituting 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 phagocytes (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).

1.1.3.2.2 A granulocytes:

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).

1.1.3.3 Thrombocytes:

The formation of thrombocyte (platelet) is called thrombopoeisis. Platelet 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.*, 2006), 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).

1.1.3.3.1 Platelet indices:-

Mean Platelet Volume (MPV):

MPV is the average volume of individual platelet 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, 2006).

Platelet cirit (PTC):

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

1.1.3.3.2 Platelet 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).

1.1.4 Complete blood count test (CBC):

A complete blood count (CBC), also known as full blood count (FBC) or full blood exam (FBE) or blood panel, is a test panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood. A CBC is routinely performed during annual physical examinations in some jurisdictions. The CBC includes determinations of the hemoglobin, hematocrit, red blood cell count, red blood cell volume; and hemoglobin content, platelet count, and white blood cell count. These measurements are provided by any of the common automated counters, including instruments manufactured by Abbott, Bayer, Beckman-Coulter, and Technicon. A CBC also helps him or her diagnose conditions, such as anemia, infection, inflammation, bruising, bleeding disorders or leukemia, acute hemorrhagic states, allergies and it is also crucial in monitoring the condition and/or effectiveness of treatment after a diagnosis is established (Dacie and Lewis, 2006).

A very important advantage of CBC analysis is its short working time (approximately 10 minutes). Besides this, there is no need for fasting and a rational amount of specimen is needed for the procedure. The widespread use of hematology analyzers (HA) has led to major improvement of cellular hematology, because of quick and accurate results found in most instances,

and now preanalytical and analytical variables should be considered first within the laboratory when spurious results from the HA are found. Preanalytical factors like venipuncture, collection of inadequate volumes of blood and storage conditions are among the most common factors that affect the CBC results. (Zandecki, *et.al*., 2007)

1.1.4.1 Physiological factors which affect outcomes of CBC test:

Red cell components: There is considerable variation in the red blood cell count (RBC) and Hb at different periods of life and there are also transient fluctuations, the significance of which is often difficult to assess. There are rapid fluctuations in the blood count of newborn babies, infants and older children. Reference ranges for preterm infants vary with gestational age. The RBC and Hct also fall, although less steeply, and the cells may become microcytic with the development of iron deficiency. The Hb and RBC increase gradually through childhood to reach almost adult levels by puberty. The levels in women tend to be significantly lower than those in men. (Dacie and Lewis, 2006). In normal pregnancy, there is an increase in erythropoietic activity and a simultaneous increase in plasma volume occurs, which overall results in a progressive decrease in Hb, Hct and RBC. There is a slight increase in MCV during the second trimester. The hematological parameters return to normal about a week after delivery. (Dacie and Lewis, 2006).In healthy men and women, Hb, RBC, Hct and other red cell indices remain remarkably constant until the sixth decade. Anemia becomes more common in those older than 70–75. Factors that contribute to the lower Hb in the elderly include renal insufficiency, inflammation, testosterone deficiency, diminished erythropoiesis, stem cell proliferative decline and myelodysplasia. (Dacie and Lewis, 2006). Optimal athletic performance depends on proper function of

many organs, including the blood. Several hematological parameters can affect or be influenced by physical activity, endurance athletes may develop so-called ‘sports anemia’. The effects of exercise must be distinguished from a form of haemolysis known as ‘runner’s anemia’ or ‘march haemoglobinuria’. Diurnal and seasonal variation changes in Hb and RBC during the course of the day are usually slight. (Dacie and Lewis, 2006).The effect of altitude is to reduce the plasma volume, increase the Hb and Hct and raise the number of circulating red cells with a lower MCV. The magnitude of the polycythemia depends on the degree of hypoxaemia. Corresponding increases occur at intermediate and at higher altitudes. These increases appear to be the result of enhanced erythropoiesis secondary to the hypoxic stimulus, and the decrease in plasma volume that occurs at high altitudes. (Dacie and Lewis, 2006). Cigarette smoking affects Hb, RBC, Hct and MCV. (Dacie and Lewis, 2006).

Leucocyte count: At birth, the total leucocyte count is high; the levels are the same as those of adults. There are also slight sex differences; the total leucocyte count (WBC) and the neutrophil count may be slightly higher in girls than in boys, and in women than in men. In women this may be related to the menstrual cycle or to the use of oral contraception. Random activity may raise the count slightly; strenuous exercise causes increases of up to 30 × 109/l, large numbers of lymphocytes and monocytes also enter the bloodstream during strenuous exercise. Epinephrine (adrenaline) injection causes an increase in the numbers of all types of leucocytes (and platelet),. Emotion may possibly cause an increase in the leucocyte count. Cigarette smoking has an effect on the leucocyte count. A moderate increase in the WBC, of up to 15 × 109/l, is common during pregnancy.

In individuals of African ancestry there is a tendency for the neutrophil: lymphocyte ratio to be reversed primarily due to a reduction in neutrophil

count. This is due to genetic rather than environmental factors. Significantly lower WBC and neutrophil counts have also been observed in Africans and Afro-Caribbean's living in Britain as well as in many African countries. 'Benign ethnic neutropenia' Elderly people receiving influenza vaccination show a lower total leucocyte count owing to a decrease in lymphocytes. (Dacie and Lewis, 2006).

Platelet count: There is a slight diurnal variation in the platelet count of about 5%; this occurs during the course of a day as well as from day-to-day. Within the wide normal reference range, there are some ethnic differences, and in healthy Afro-Caribbean's and Africans platelet counts may on average be 10–20% lower than those in Europeans living in the same environment. There is also a gender difference; thus, in women, the platelet count is about 20% higher than in men. A decrease in the platelet count may occur in women at about the time of menstruation. In the first year after birth the reference range for the platelet count is higher than the adult reference range. Strenuous exercise causes a 30–40% increase in platelet count. (Dacie and Lewis, 2006).

1.1.4.2 Pathological factors which affect outcomes of CBC test:

Table 1.3: Explain what the result for each component of the CBC may mean: (Greer, *et .al,* 2009) and (McPherson and Pincus, 2011)

Test	**Examples of causes of high result**	**Examples of causes of low result**
White Blood Cell Count (WBC)	Known as leukocytosis infection (most commonly bacterial or viral), inflammation, leukemia, myeloproliferative neoplasms, allergies, asthma. and tissue death (trauma, burns, heart attack)	known as leucopenia bone marrow disorders or damage, autoimmune conditions ,severe infections (sepsis), lymphoma or other cancer that spread to the bone marrow ,dietary deficiency, and diseases of immune system (likes HIV/AIDS)
Absolute neutrophil count, % neutrophils (Neu, PMN, polys)	known as neutrophilia acute bacterial infections, inflammation, trauma, heart attack, burns and certain leukemia's (likes chronic myeloid leukemia), and Cushing syndrome	known as neutropenia severe, overwhelming infection (sepsis) , autoimmune disorders , dietary deficiency , reaction to drugs, Immunodeficiency, myelodysplasia , bone marrow damage and cancer
Absolute lymphocyte	known as lymphocytosis acute viral infections (likes, chicken	known as lymphocytopenia autoimmune disorders, (likes

count, % lymphocytes (Lymph)	pox, cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes, rubella) certain bacterial infections (like pertussis (whooping cough), tuberculosis (TB)) toxoplasmosis , chronic inflammatory disorder (like ulcerative colitis), lymphocytic leukemia, and lymphoma stress (acute)	lupus, rheumatoid arthritis) ,infections (like HIV, viral hepatitis, typhoid fever, influenza) bone marrow damage (likes chemotherapy, radiation therapy) , and corticosteroids
Absolute monocyte count, % monocytes (Mono)	chronic infections (likes tuberculosis, fungal infection) infection within the heart (bacterial endocarditic) collagen vascular diseases (like, lupus, scleroderma, rheumatoid arthritis, vasculitis) monocytic or myelomonocytic and leukemia (acute or chronic)	usually, one low count is not medically significant. repeated low counts can indicate: bone marrow damage or failure hairy cell , leukemia and A plastic anemia
Absolute eosinophil count, % eosinophils (Eos)	asthma, allergies such as hay fever drug reactions ,parasitic infections, inflammatory disorders (celiac disease, inflammatory bowel disease)	Numbers are normally low in the blood. one or an occasional low number is usually not medically significant.

	some cancers, leukemia or lymphomas and Addison disease	
Absolute basophil count, % basophils (BASO)	rare allergic reactions (hives, food allergy) inflammation ,(rheumatoid arthritis, ulcerative colitis) and uremia	as with eosinophils, numbers are normally low in the blood; usually not medically significant.
Red Blood Cell Count (RBC)	known as polycythemia dehydration , lung (pulmonary) disease kidney or other tumor that produces excess erythropoietin ,smoking living at high altitude ,genetic causes (altered oxygen sensing, abnormality in hemoglobin oxygen release) and polycythemia vera—a rare disease	known as anemia acute or chronic bleeding ,RBC destruction (likes hemolytic anemia, etc.) ,nutritional deficiency (like, iron deficiency, vitamin B_{12} or folate deficiency) ,bone marrow disorders or damage, chronic inflammatory disease and chronic kidney disease
Hemoglobin (Hb)	usually mirrors RBC result	usually mirrors RBC results, provides added information
Hematocrit (Hct)	usually mirrors RBC results; most common cause is	usually mirrors RBC results

	dehydration	
MCV	indicates RBCS are larger than normal (macrocytic), like in anemia caused by vitamin B_{12} or folate deficiency, myelodysplasia, liver disease, hypothyroidism	Indicates RBCS are smaller than normal (microcytic); caused by iron deficiency anemia or thalassemia.
MCH	Mirrors MCV results; macrocytic RBCS are large so tend to have a higher MCH.	Mirrors MCV results; small red cells would have a lower value.
MCHC	Increased MCHC values (hyperchromia) are seen in conditions where the hemoglobin is more concentrated inside the red cells, such as autoimmune hemolytic anemia, in burn patients, and hereditary spherocytosis, a rare congenital disorder.	May be low when MCV is low; decreased MCHC values (hypochromia) are seen in conditions such as iron deficiency anemia and thalassemia.
RBC Distribution Width (RDW)	Indicates mixed population of small and large RBCS; young RBCS tend to be larger, for example, in iron deficiency anemia or pernicious anemia,	Low value indicates uniformity in size of RBCS.

	there is high variation (anisocytosis) in RBC size (along with variation in shape – poikilocytosis), causing an increase in the RDW.	
Platelet Count (Plt)	known as thrombocytosis: cancer (lung, gastrointestinal, breast, ovarian, lymphoma) rheumatoid arthritis, inflammatory bowel disease, lupus iron deficiency anemia, hemolytic anemia and myeloproliferative disorder (likes essential thrombocythemia)	known as thrombocytopenia: viral infection (mononucleosis, measles, hepatitis) rocky mountain spotted fever platelet autoantibody drugs (acetaminophen, quanidine, sulfa drugs) cirrhosis autoimmune disorders sepsis, leukemia, lymphoma , and myelodysplasia
(MPV) Mean Platelet Volume	Indicates a high number of larger, younger platelet in the blood; this may be due to the bone marrow producing and releasing platelet rapidly into circulation.	Indicates average size of platelet is small, low MPV may mean that a condition is affecting the production of platelet by the bone marrow.

1.1.5 Toombak:

1.1.5.1 Definition of toombak:

In the Sudan, snuff locally known as toombak was introduced approximately 400 years ago. It is always processed into loose moist form, and its use widespread in all over the country. Tobacco used for manufacturing of toombak is of species *Nicotana rustica*. The fermented ground powder is mixed with an aqueous solution of sodium bicarbonate (Idris, *et.al,* 1998a). Introduction of this tobacco plant to the Sudan was attributed to a Quranic (Islamic) teacher who came to the Sudan, either from Egypt, Timbuktu of Mali or Morocco. It has also been suggested that toombak was introduced to the Sudan from Turkey or Arabia. The commercial names for toombak include, El-Sanf (of high quality) Wad Amari (according to the person who was believed to have introduced it) and Sultan Elkaif (the power to improve one's state of mind). (Badie, 2007)

Tobacco is primarily consumed in the Sudan in two forms oral snuff and Cigarettes. Oral snuff is consumed as twice as cigarettes and named toombak in the local language is home-made from finely ground leaves of *Nicotana rustica*. Tobacco species with especially higher content levels of alkaloid (nicotine, Anabasine, nornicotine) than *Nicotana tobacum* used for cigarettes which is a prime factor for popularity of tobacco (Idris, *et. al*.1995a).

Smokeless tobacco product (toombak) has been used in the Sudan for centuries and is widespread, especially in the northern, eastern and central parts (Idris, *et .al*., 1994). The use of toombak is particularly common among the Gaalen and Shaigia tribes who reside these regions (EL-Besheir, *et.al*., 1989).

1.1.5.2 Prevalence of toombak:

The prevalence of toombak use among the male population aged 18 years and older in Sudan was 34%. The prevalence of toombak use among the male population aged 18 years and older was significantly higher in the rural than in the urban areas (35% vs. 24%). The highest rates of toombak use were found in rural areas among the male population aged 30 years and older (Idris, *et.al*, 1998b). In that study it was clearly documented that the prevalence of toombak use was as high as 12.6% in the entire population of the Nile State (age4+ years).This prevalence was sevenfold higher than the estimates suggested previously (.El-Besheir, *et.al*, 1989), and was at least two fold higher than any reported rates of oral snuff used from high prevalence areas in North America, Sweden, Norway, Nigeria, and South Africa. Nasal snuff as practiced in the United States, Europe, and some areas in Africa and the practice of chewing tobacco as found in Asia (Marcus, *et.al*., 1989, Hoover and Hartsfild, 1990 and Schei, *et.al*., 1990). Toombak usage was confined almost exclusively to males. Little is known about factors that contribute to this gender difference. Some of the differences found might reflect underreporting by females, since it is generally accepted by the people of the Sudan that females tend to deny these habits while the use of these products by males is perceived as more socially acceptable. The continued toombak use until old age, the high nicotine content, and the use of natron (sodium bicarbonate) in its processing all suggest that toombak is a highly addictive substance (Idris, *et.al*., 1991).

1.1.5.3 Botany of tobacco plant:

The genus *Nicotiana* is classified among the family *Solanaceae* which comprises about 100 species. The most famous species is the largely

cultivated Virginia. *Tobacco, Nicotiana tobacum, Turkish tobacco* and *Nicotiana rustica* (Broun and Massey, 1929).

Tobacco is believed to be a native of tropical America and was cultivated and used by native inhabitants before the discovery of America. It is the one of the few major contributions to civilization which the new world can claim. The first who used tobacco were the Indian of North and South America and Spread to other countries France 1556, England 1565 and from these countries to the different parts of the world (Hussain, 1984).

Nicotiana rustica, it is a semi desert plant, grows in different areas in the Sudan but mainly in Darfur at the Western region. The herb is up to four feet high. Leaves pediculate ovate obtuse at the apex, sometimes subcordate at the base, up to one feet high long glandular pubescent. Flowers are greenish yellow, in terminal sub paniculate. Racemes with or without bract. Capsule sub loose slight longer than the calyx (Broun and Massey, 1929).

1.1.5.4 Chemical composition of tobacco:

Natural tobacco contains at least 30-50 different compounds. Furthermore, smokeless tobacco may be enhanced by flavoring agents, added in the form of plant extracts and /or as chemicals (Roberts, 1988). Among 23 tumorigenic agents in smokeless tobacco (Wynder, *et.al.*, 1967), are Volatile aldehydes and N-nitrosamines, Nitrosamine acids, lactones, poly nuclear Aromatic hydrocarbons pyrine, primanly benzo, and carbonates, certain metals And the emitters, polonium-210 and uranium -235 and -238. Of these Nnitrosonornicotine (NNN) and 4- (methynitrosamine) -1-(3-pyridyl-1-butanone (NNK) are the predominant carcinogens in smokeless tobaccos. Sudanese Toombak contains high concentration of TSNA, due to the use of *N.* rustica its preparation. NNN and NNK levels in N. rustica have been reported to be much higher than *N*. tobacum (Bhide, *et.al.*, 1987). The active ingredient

in tobacco is alkaloids of naturally occurring compound Containing nitrogen and having the properties of an amine base, they have Dramatic effects on the human system (Hammond, 1962). It was first isolated from genus Nicotana in 1828, nicotine is a Colorless oily liquid alkaloid, and it is considered on the most toxic drugs known to human, a dose of 60 mg is lethal in a few minutes (Pavia, *et.al*, 1976).

Hussain, (1984) reported that nicotine constitutes 0, 9 to 3, 8% of Nicotiana tobacum and between 7-12% of Nicotianarustica. Nicotine is an organic compound, an alkaloid that is naturally found in the Tobacco plant. Although it is present throughout all the plant, it can be found in particularly high concentrations in the leaves, which contain 0.3- 5% of dry weight. Nicotine is a mind-altering substance Liquid in its pure state; it turns brown in contact with the air. It is a powerful neural poison. In low concentrations, the substance acts as a stimulant and main factor responsible for the dependence-forming properties of tobacco smoking. Nicotine molecule in small doses, it is a stimulant: which increases activity, Concentration and memory. It also increases heart rate and blood pressure and reduces appetite. In high doses it causes nausea and vomiting.

1.1.5.5 Cultivation of toombak in Sudan:-

Toombak grows in silt or sandy soil which receives heavier rain falls in the North West of the Sudan, after the end of the rain season September/ October Toombak is planted during the months November /December and never irrigated. At first it is broadcasted in the farm and then transferred to new areas which are called Makhamas. Harvesting starts in the months February/March when the Leaves turn yellow and brownish spots start appearing which are called the small pox Stage. Harvested leaves are left in the field for uniform drying, tied into bundles, moistened by sprinkling water

and stored for fermentation for couple of weeks at temperature ranging from 30 to 45°C during which bundles are separated for uniform drying during the months April/May. Tobacco leaves are ground and stored for a year for ageing (Idris, 1992).

1.1.5.6 Processing of toombak:-

Tobacco leaves after cutting from the trees are dried in a big basket to ferment and the color changes from yellow to brown after the fermentation process. The leaves will be milled using electrical miller. The product is milled to different particles size this is mainly related to consumer taste consideration. Since in Eastern part of Sudan people prefers the coarse product while in Khartoum and Central region, they prefer the fine or powdered product. The milling process is done in the same areas of cultivation in Sudan. Most of milling machines are centered in El Fashir town in Darfur province. Processing of toombak for sale is usually carried out manually entombed shops by toombak vendors. It is performed by preparing four parts of a coarse powder of dried Toombak leaves in a bowl and in another the concentrate of Natron (sodium Bicarbonate) (1:4 Natron and water) is added gradually in small amounts to the tobacco (Idris, 1992). While adding the solution, the product is mixed vigorously by both hands and concurrently tested by sensation of the fingers tips until it becomes moist and hardened. The output is then transferred to special air tight tin containers which are then covered firmly for about 2 hour thereafter the Product becomes ready for sale or use. Before buying users generally ask for a bit to smell or test, because the aroma and test decide the quality rank of the product. (Badie, 2007)

Currently, toombak is sold in small plastic bags each taking about 100g. Some toombak users carry round or box shaped tin cans in his pocket named hookah

and are similar to plastic bags though some people use king size. Hookah is still used by some people and it make an indentation in the pocked of user, thus one can easily guess and identify (Badie, 2007).

1.1.5.7 The habit of toombak in the Sudan:

Toombak can be bought from innumerable shops in the market, and the product is advertised extensively at points of sale where vendors tend to use commercial names to attract buyers. The habit of toombak dipping is practiced by taking a small portion from the bag or hookah with the therefore -fingers, usually of the right hand, putting it in the palm of the left hand, and manipulating it by the thumb and middle fingers of the right hand until it forms a ball called (Saffa) which is of about 10g in weight. The Saffa is not chewed but dipped and retained between gum and lip or cheeks or floor of mouth, and sucked slowly for about 10-15 minutes. Generally, men prefer dipping between the lower lip and gum, while women prefer dipping between cheeks and gum. The dipping continues for a period ranging from a few minutes to several hours, until the Saffa becomes bland. Men periodically spit the insoluble debris that is freed from the bulbous and the saliva which is secreted during toombak use, whereas women retain the saffa without spitting because of social unacceptability. The mouth is usually rinsed with water after the quid is removed. The toombak quid is sometimes retained in the mouth during sleep (Idris, 1992).

1.1.5.8 Absorption of nicotine in the body:

Nicotine absorption occurs at different parts of body chiefly in the mucosal tissue of the mouth, respiratory tract, intestine and skin (Hussain, 1984). There are a few studies that have directly examined the effects of pH

on nicotine absorption, Beckett, *et.al*, 1972 found very little buccal absorption of nicotine from tobacco when the pH was 5.5. Ten percent absorption at pH of 7, and about 30% at pH of 9.0.

Henningfield, *et.al.,* 1990 found that rinsing with acidic beverages such as coffee or cola before chewing nicotine polacrilex nearly eliminated nicotine absorption. These results indicate that pH is an important determinant of buccal absorption of nicotine.

Henningfield, *et.al.,* 1995 found that the nicotine content of six moist snuff products ranged from (7.5mg/g to 11.4mg/g) and that the pH of these products ranged from (6.9 to 8.6). The pH of the snuff is important because nicotine most readily crosses the oral mucosa in the unionized form. The degree to which nicotine is unionized depends on the higher pH levels (more alkaline).

The rate of absorption is highest when the snuff is first placed in the mouth and plasma concentration continued to rise until the snuff was removed from the mouth. Absorption continued even after the snuff was removed, presumably because of the slow release of nicotine from the mucosa into the plasma or absorption of swallowed nicotine in the gut. (Badie, 2007)

1.1.5.9 Metabolism of nicotine in the body:

When nicotine is absorbed, it is immediately distributed into different parts of the body brain, lungs, liver, intestine, spinal cord and adrenal gland. Liver is the site of breaking down nicotine into harmless compounds which pass in urine with small amount of unmetabolized nicotine (Hussain, 1984).

1.1.5.10 Physiological and pharmacological effects of nicotine in the body:

Large amounts of nicotine are delivered rapidly to the blood stream during use of moist snuff. In fact venous nicotine concentrations are higher

than those which have been observed following cigarette smoking. (Badie, 2007)

Benowitz, *et.al.*, 1988 found that average peak blood nicotine concentration increased 14.3 ng/ml after smoking one cigarette or using 2.5g moist snuff for 30 minutes. Four brands of moist snuff were tested have comparable nicotine content (11.4, 10.4 and 11.4mg/g respectively), but produced different pH values in suspension (8.6, 7.6, and 7.5 respectively) (Henningfield, *et.al.*, 1995), these study confirm that the pH of these products in suspension is a significant factor in determining nicotine bio viability and increasing of heart rate after moist snuff administration is associated with the nicotine levels attained by each product. The heart rate increases during the first 15 minutes of administration and then declined after about 15 minutes of administration and despite continued increases in nicotine plasma concentration. The role of nicotine in producing these effects has been established by studies of direct administration. In general, the responses are consistent with activation of the sympathetic nervous system. Cardiovascular effects include heart rate acceleration (10 to 20 beats/min) and increased blood pressure (5 to 10 mmHg), similar to the effects of cigarette smoking. Nicotine also increases the circulating levels of catecholamines and free fatty acids, which may contribute to the increased level of total cholesterol and decreased levels of high-density lipoprotein cholesterol that are found in habitual cigarette smokers. Inhibition of prostacyclin synthesis and other effects on platelet may enhance coagulation. (Benowitz, 1988). High level of nicotine in blood stream produces nausea, vomiting, and cardiovascular diseases (Asplund, 2001), Exposure to high concentrations of nicotine has adverse effects on a number of physiological and biochemical processes involved in atherosclerosis (Kilaru, *et.al*, 2001). In addition, the evidence that smokeless tobacco use may

increase the risk of cardiovascular disease and cancers of the larynx, esophagus, and other sites, as well as disease of gingival and periodontal tissue. Recent data suggest that some forms of smokeless tobacco may increase the risk of dental caries (Tomar, and Mini, 1998). The increased popularity of toombak use in recent years seems to be due to it satisfying some psychosocial, pharmacological, economic and social demands. Regarding the psychosocial demands, toombak helps to alter mood, and ambiguously helps both concentration and relaxation and distraction. That is provided by both the intervals of preparation of the saffa and the dipping. The pharmacological effects are mainly attributed to nicotine that is a powerful pharmacological agent that changes the cardiovascular, neural, endocrine, and muscle function and induces effects in the gastrointestinal tract. The cardiovascular changes include increased heart rate, blood Pressure and decrease in skin temperature due to vasoconstriction in the extremities. The nervous effects in the brain and the peripheral nervous system are associated with changes in electrical cortical activity likes induction of both stimulation and relaxation. In the gastrointestinal tract, nicotine stimulates the parasympathetic autonomic ganglia and brain stem, causing the release of pharmacologically active substances which may produce nausea, vomiting and occasionally diarrhea, therefore, it is now accepted that tobacco causes physical dependence addiction and habituation (Huhtassaari, 1999).

1.1.5.11 Toombak and oral cancer:

Toombak has been known to play a major role in the etiology of oral cancer in the Sudan (Idrisb, *et.al*., 1995). It contains at least 100-fold higher concentrations of the carcinogenic factor tobacco specific N-nitrosamines compared with American and Swedish commercial snuff brands (Idris, *et.al.*,

1994). A recent study showed that toombak induces DNA damage and cell death in normal human oral cells more than the Swedish snuff (Costea, *et.al*, 2014) .Use of Toombak has been etiologically linked to various oral diseases, such as periodontal diseases, mucosal lesions and may eventually lead to tooth loss (Robertson, *et.al.*, 1997 and Anand, *et.al*, 2012)**..** Although a high relative frequency of oral cancer in the Sudan has been observed since the years 1959 and 1963 (Hickey, 1959 and Lynch, *et.al*., 1963), the earliest observation on the association between oral cancer and use of toombak was reported in 1980 .Much later, it was found that 81% (50/62) of patients with oral Squamous cell carcinoma (SCC) from the Sudan used toombak (El-Besheir, *et.al.*, 1989).

1.2 Rationale:

Prevalence of toombak use is increasing among male population it was found to be 35% in rural and 24% in urban areas and the highest rate of toombak use found in aged 18 years and older and the toombak use found to be associated with mouth cancer and cardiovascular disease.

There is scarcity of data concerning the effect of toombak dipping on CBC, so this work was done to add the present knowledge about this.

1.3 Research Objectives:

1.3.1 General objective:

To estimate the effects of toombak dipping on CBC.

1.3.2 Specific objectives:

- To compare complete blood count of toombak dippers (cases) with non-toombak dippers (controls).
- To determine some characteristic of the toombak dippers.
- To study the effect of duration of toombak dipping, age of dippers and the number of dipping / day on CBC.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study design:

This is an analytical case control community based study conducted to estimate the effect of toombak dipping on complete blood count (CBC) of the Sudanese dippers in Khartoum State.

2.2 Study area:

This study was carried out during the period from September 2018 to April 2019 in Khartoum State.

2.3 Study population:

The participants were 35 healthy toombak dippers as cases and 50 healthy non-toombak dippers volunteers as controls, all the participants were chosen by non-probability volunteers sampling and they were free from diseases or medication therapy in the last month before sample collection.

2.3.1 Inclusion criteria:

Sudanese toombak dippers.

2.3.2 Exclusion criteria:

-Healthy non Sudanese dippers.

-Toombak dippers males who are smokers, alcohol drinkers or shisha smokers

-Male with illness which may affect the hematological level likes (anemia, leukemia, liver disease, kidney disease, mal nutritional, inhaled steroid, and bleeding disease)

-Exclude any toombak dippers who receive medication or transfusion recipient less than 6 month.

2.4 Data collection:

Data were collected using a personal interview questionnaire and laboratory investigation, the questionnaire included some information of the participants

likes: age, marital status, education level, occupation, duration of dipping, numbers of dipping per day, site of dipping and some of health problems.

2.5 Blood collection:

Venous blood was collected from all the participants. Using of venipuncture via the antecubital vein to collect 2.5 ml of blood from each volunteer. Blood collected in test tubes contains K_3 Ethylene Di-amine Tetra-acetic Acid (K_3EDTA) as anticoagulant. Each sample was mixed gently and thoroughly to prevent cell lyses and clotting of blood. Complete blood count (CBC) was determined within 2 hours after collection using a hematological analyzer (Hemolyzer 3 Pro, manufactured by Analyticon corporation- Germany).

2.6 Laboratory investigation:

Complete blood count was investigated using hematological analyzer a three-part auto analyzer of parameters: WBC, LYM, MID, GRA, RBC, MCV, HCT, HGB, MCH, MCHC, RDW, PLT, MPV, PCT, and PDW.

2.6.1 Operating principles of analyzer:

The impedance method (a.k.a. Coulter method) counts and sizes cells by detec ting and measuring changes in electrical impedance when a particle in a condu ctive liquid passes throuh a small aperture. Each cell passing through the apert ure, there is a constant DC current flowing between the external and internal el ectrodes causes some change in the impedance of the conductive blood cell su spension. These changes are recorded as increases in the voltage between the e lectrodes. The number of pulses is proportional to the number of particles. The intensity of each pulse is proportional to the volume of that particle. The volu me distributions of the cells are displayed on numbers or diagrams.

2.6.2 Quality control of the machine:

All quality control of the machine done in instructed manner. The daily, weekly and monthly maintenance and calibration used to ensure quality assurance. Then before using the apparatus one of the last day samples was re-analyzed for data check**.**

2.6.3 Procedure:

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

2.6.4 Reagents and materials:

All reagents were cyanide-free and have extended shelf life.

Reagents	**Name**
Isotonic Diluent	Hemolyzer-Diluent
Hemolysing Agent	Hemolyzer-3-Lyser
Cleaner	Hemolyzer-3-Cleaner
Contr./Caliber./Cleaner	**Name**
Control	Hemolyzer-3-Control Set (3 Level)
Control /Calibrator	Hemolyzer-3-Control Normal (Level N)
Cleaner (external)	Hemolyzer-Hypocleaner cleaning solution

2.7 Statistical Analysis:

The data obtained were analyzed by Statistical Package of Social Science (SPSS.version -25) software program. The characteristics of the study groups (age, marital status, education level, occupation, duration of dipping, numbers of dipping / day, site of dipping, daily cost of dipping, and some of health problems) were presented by percentage .

Effect of toombak dipping on CBC was determined by independent sample T-test. One-way ANOVA was used to show the effect of age, duration of dipping and numbers of dipping daily on complete blood count. Significance level was set as $P. < 0.05$.

2.8 Ethical consideration:

The study was approved by the Medical Laboratory College Committee −SUST. A written 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 than this study.

CHAPTER THREE
RESULTS

3.1 Characteristics of the dippers population:

Unfortunately, among health-damaging habits, cigarette smoking and toombak dipping are socially accepted behaviors among adults in Sudan. Both cigarettes and toombak are cheap, openly advertised, and sold in small shops on the streets. Most of the elders' dipper who was asked about the motivation factors to dip; said that is a traditional habit, other dippers like student said that they started dipping as challenge with their friends, watched parents dipping and in boring of free times.

In Fig (3.1), the distribution of toombak dippers according to their ages is shown the highest percentage (46%) was recorded by the age group <20 years old and the lowest percentage (11%) by the group of >40 years.

In Fig (3.2), the single males reported highest percentage (91%) while the married male the percentage was (9%).

In Fig (3.3), the secondary education reported highest percentage (51%) in contrasts of primary (11%) and high education (37%).

In Fig (3.4), the students register the highest percentage (37%) of toombak use.

In Fig (3.5), the duration of <5 years reported highest percentage (51%) among dippers.

In Fig (3.6), <15 number of dipping per day is the highest percentage (51%) of toombak dippers.

Fig (3.7), shows the distribution of toombak dippers according to site of dipping in mouth, the upper lip occupy the highest percentage (54%) between other sites of mouth.

Fig (3.8) shows the distribution of daily cost of dipping.

The health problems found in toombak dippers displayed that the high frequency of the mouth problems are registered in the ulceration of gums ,color change of teeth and dryness of lips. Table (3.1)

3.2 Effect of the toombak dipping on complete blood count:

In table (3.2) the toombak dipping affected CBC and caused significant ($P \leq 0.05$) increase compared with the control group in (WBCS, RBCS, HCT, RDW and PLT), and a significant decrease compared with control group in (MCH, MCHC and MPV) and no effect in (GRA, LYM, MID, HB, MCV, PCT and PDW).

3.3 Effect of the toombak dippers age on complete blood count:

Table (3.3) shows significant variations on the values of LYM among the different age groups while the other parameters were not affected.

3.4 Effect of the duration of toombak dipping on complete blood count:

All blood parameters were not affected by the duration of toombak dipping on complete blood count. Table (3.4).

3.5 Effect of the numbers of dipping / day on complete blood count:

All blood parameters were not affected by the number of dipping / day on complete blood count. Table (3.5)

Characteristic of the dippers populations

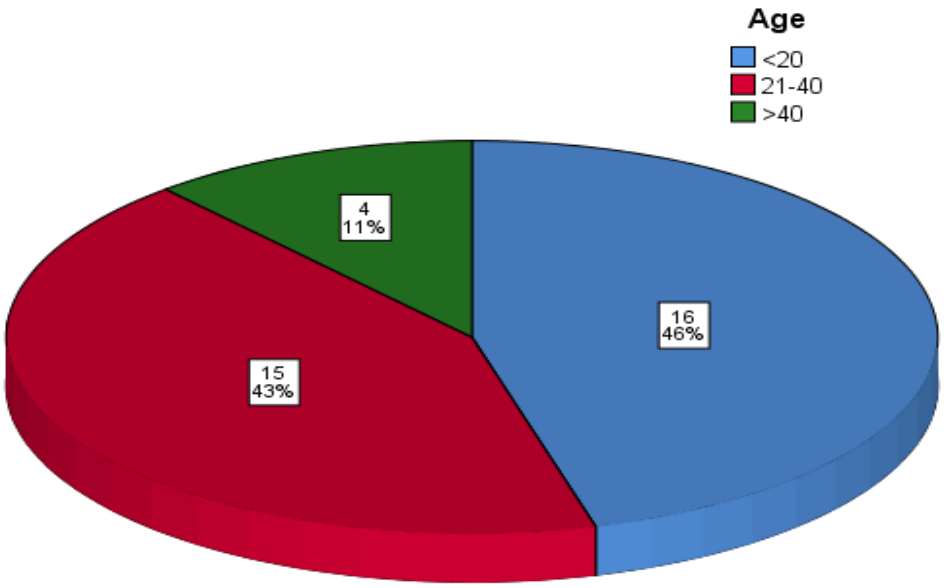

Fig 3.1: Distribution of toombak dippers according to their age.

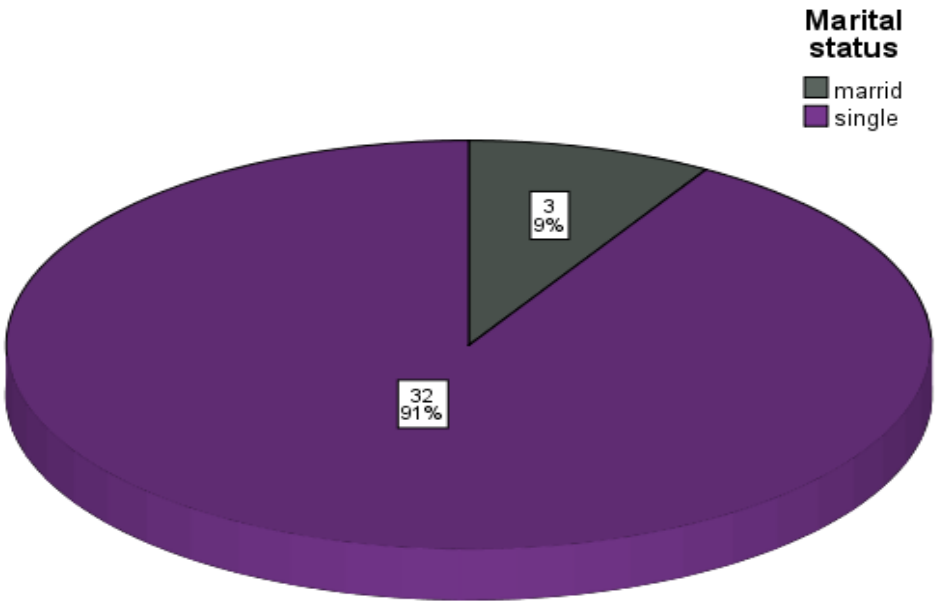

Fig 3.2: Distribution of toombak dippers according to their marital status.

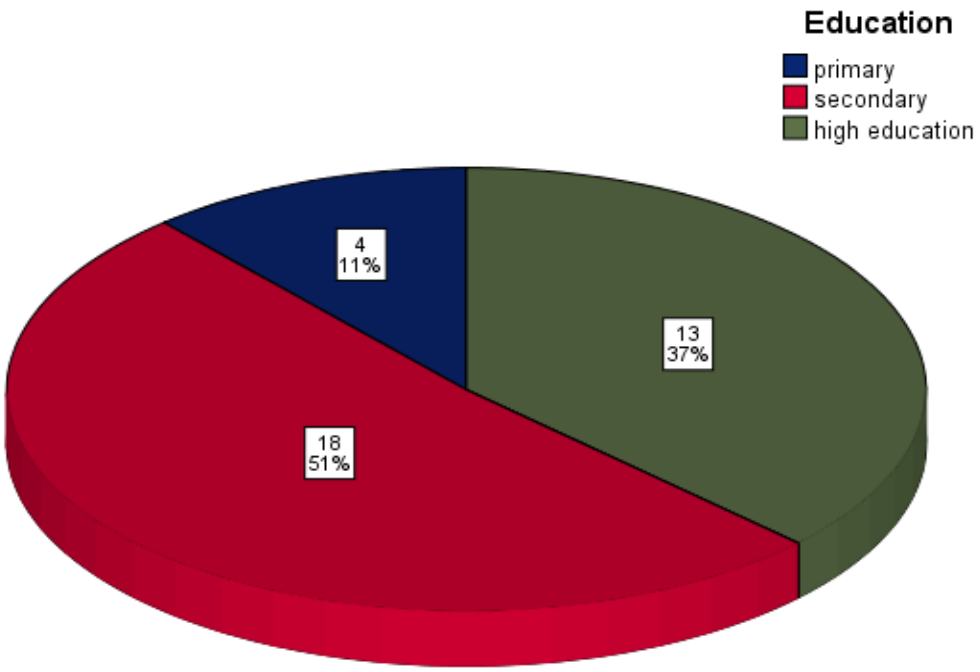

Fig 3.3: Distribution of toombak dippers according to their education level.

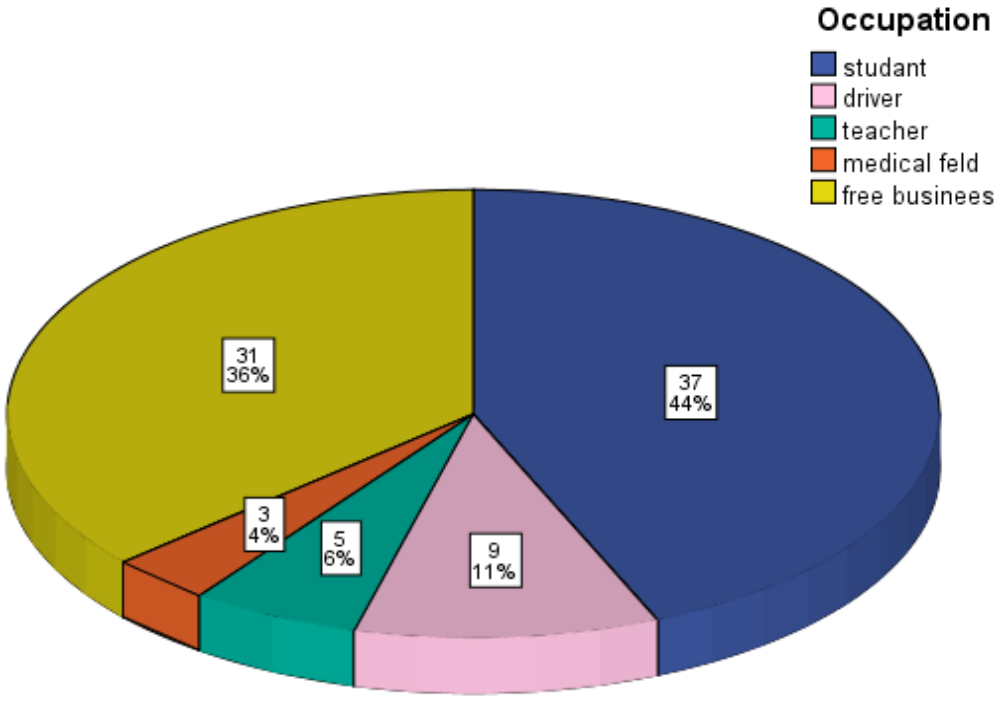

Fig 3.4: Distribution of toombak dippers according to their occupation**.**

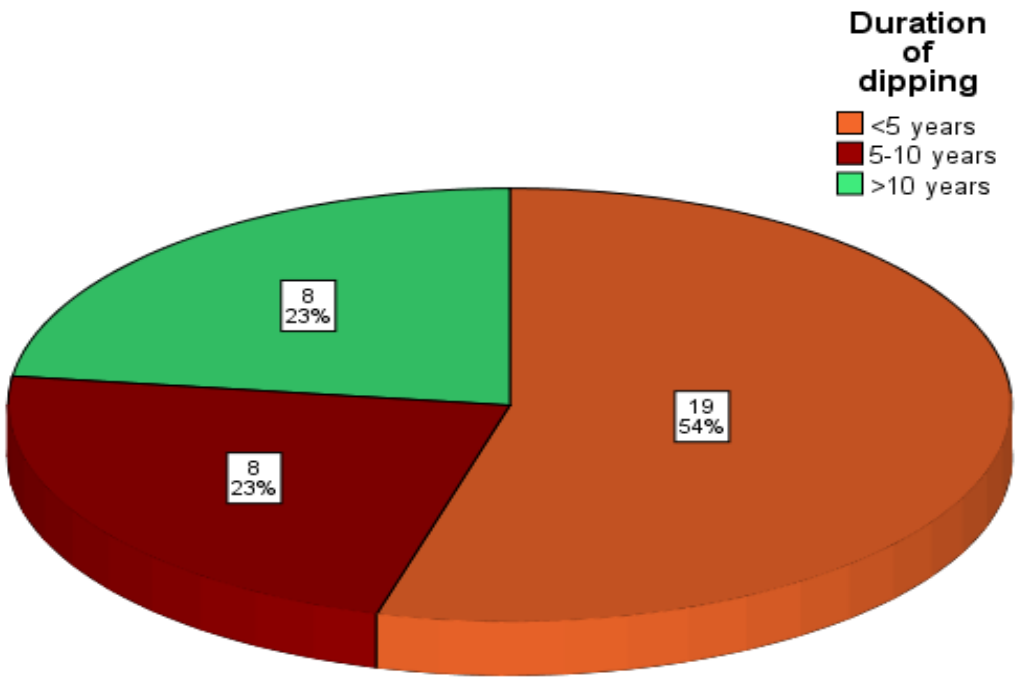

Fig 3.5: Distribution of toombak dippers according to the duration of dipping.

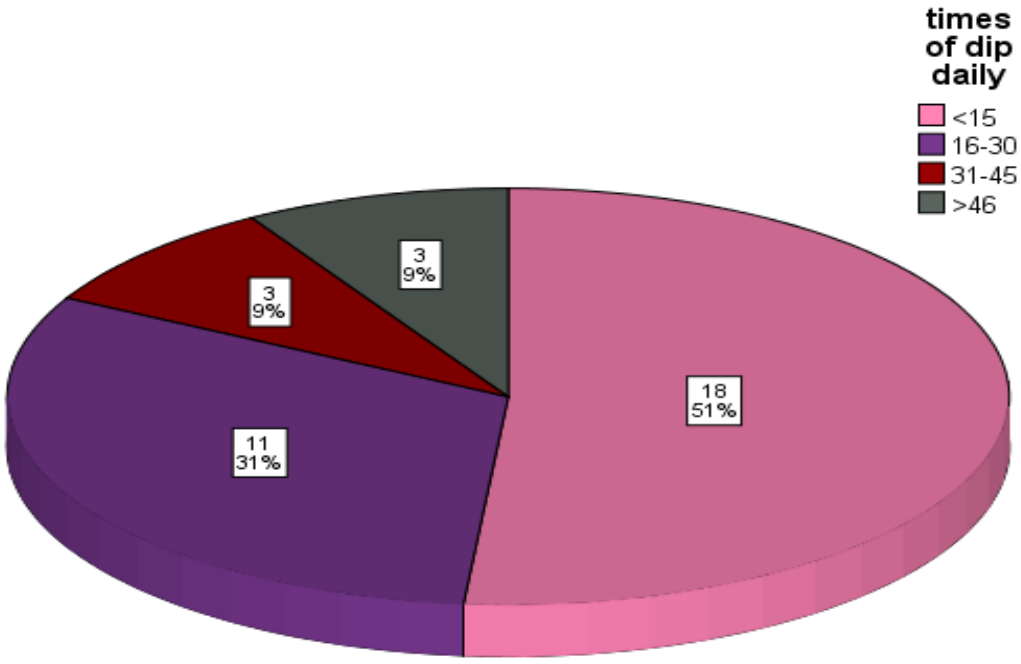

Fig 3.6: Distribution of toombak dippers according to the number of dipping / day.

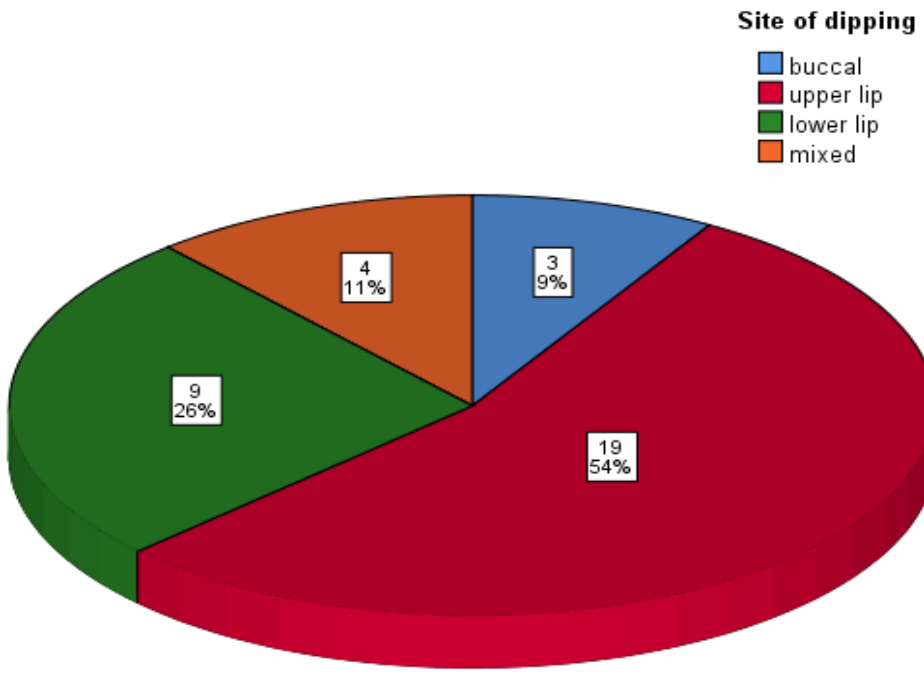

Fig 3.7: Distribution of toombak dippers according to the site of dipping.

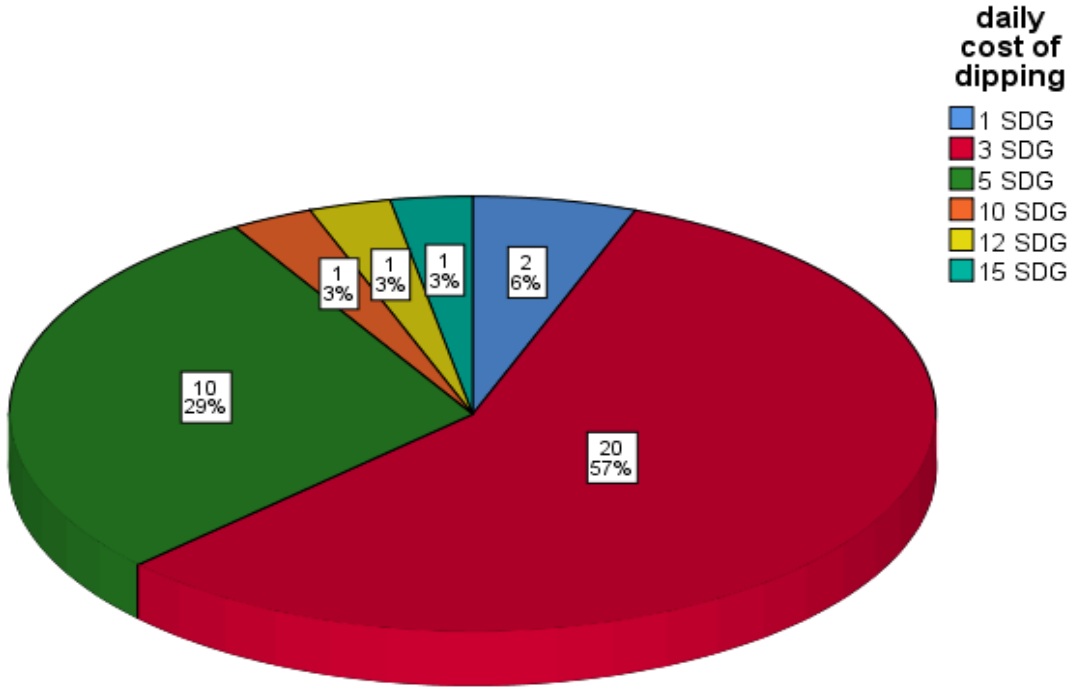

Fig 3.8: Distribution of daily cost of dipping.

Table 3.1: Distribution of toombak dippers according to the dippers health problems**.**

Health problems				Frequency	Percent
Shiver	Get a shiver			16	45.7%
	No shiver			19	54.3%
appetite affected	Decreased appetite			8	22.9%
	Not affected			27	77.1%
Mouth problems	**tongue problems**	changes in color	yes	3	8.6%
			no	32	91.4%
		Sores	yes	3	8.6%
			no	32	91.4%
	gums problems	Gingivitis	yes	6	17.1%
			no	29	82.9%
		Ulceration	yes	31	88.6%
			no	4	11.4%
		discoloration	yes	19	54.3%
			no	16	45.7%
	teeth problems	tooth decay	yes	7	20.0%
			no	28	80.0%
		Toothache	yes	5	14.3%
			no	30	85.7%
		color change	yes	18	51.4%
			no	17	48.6%
	lips problems	Crack	yes	15	42.9%
			no	20	57.1%
		Ulceration	yes	13	37.1%
			no	22	62.9%
		dryness	yes	23	65.7%
			no	12	34.3%

Table 3.2: Effect of toombak dipping on complete blood count.

Parameter	Toombak dippers Mean ±SD	Non-toombak dippers Mean ±SD	P value
WBC x10^9/l	6.4 ±1.83	5.7 ±92	**.012**
LYM x10^9/l	2.5 ±.68	2.8 ±.76	.081
MID x10^9/l	.6 ±.19	.6 ±.16	.482
GRA x10^9/l	3.3 ±1.46	3.9 ±1.31	.055
RBC x10^9/l	5.5 ±.83	5.1 ±.41	**.010**
HCT (%)	45.9 ±6.32	43.2 ±3.69	**.018**
HGB (g/dl)	14.8 ±1.55	14.7 ±1.03	.666
MCV (fl)	84.7 ±5.95	85.2 ±4.38	.692
MCH (pg)	27.4 ±3.56	28.7 ±1.89	**.033**
MCHC (g/dl)	32.5 ±3.17	34.0 ±.96	**.020**
RDW (%)	16.0 ±1.23	13.6 ±1.24	**.000**
PLT x10^9/l	290.4±100.96	246.4 ±53.27	**.011**
PCT (%)	.2 ±.10	.2 ±.05	.566
MPV(fl)	8.6 ±.98	10.2 ±1.14	**.000**
PDW (%)	40.7 ±4.39	40.4 ±2.10	.631

Significance level ($P \leq 0.05$)

Table 3.3: Effect of toombak dipping on complete blood count according to the dippers age.

Components	Age	N	Mean± SD	p. value
WBC x10^9/l	<20	16	6.8± 1.81	.492
	21-40	15	6.3± 1.76	
	>40	4	5.6± 2.31	
LYM x10^9/l	<20	16	2.9± .75	**.028**
	21-40	15	2.2± .47	
	>40	4	2.3± .60	
MID x10^9/l	<20	16	.6± .19	.470
	21-40	15	.6± .18	
	>40	4	.6± .22	
GRA x10^9/l	<20	16	3.4± 1.47	.734
	21-40	15	3.4± 1.44	
	>40	4	2.8± 1.76	
RBC x10^9/l	<20	16	5.7± 1.13	.460
	21-40	15	5.3± .47	
	>40	4	5.2± .30	
HB(g/dl)	<20	16	14.7± 1.71	.351
	21-40	15	15.1± 1.06	
	>40	4	13.8± 2.38	
HCT(%)	<20	16	47.5± 8.20	.223
	21-40	15	45.2± 2.74	
	>40	4	41.6± 6.39	
MCV(fl)	<20	16	84.3± 5.07	.771
	21-40	15	84.6± 6.61	
	>40	4	86.8± 7.89	

MCH(pg)	<20	16	26.8± 4.43	.476
	21-40	15	28.2± 2.45	
	>40	4	26.5± 3.30	
MCHC(g/dl)	<20	16	31.6± 4.53	.288
	21-40	15	33.4± .69	
	>40	4	32.9± .94	
RDW(%)	<20	16	15.9± 1.10	.407
	21-40	15	15.8± .93	
	>40	4	16.8± 2.47	
PLT x10^9/l	<20	16	329.1± 130.86	.102
	21-40	15	262.8± 44.02	
	>40	4	238.8± 74.12	
PCT(%)	<20	16	.3± .13	.099
	21-40	15	.2± .04	
	>40	4	.2± .04	
MPV(fl)	<20	16	8.6± .77	.332
	21-40	15	8.4± 1.09	
	>40	4	9.3± 1.30	
PDW(%)	<20	16	41.5± 6.04	.482
	21-40	15	39.6± 2.18	
	>40	4	41.4± 2.13	

Significance level ($P \leq 0.05$)

Table 3.4: Effect of toombak dipping on complete blood count according to the duration of dipping.

Components	Duration of toombak use	N	Mean±SD	P. Value
WBC x10^9/l	<5 years	19	6.4± 1.82	.592
	5-10 years	8	6.0± 1.56	
	>10 years	8	7.0± 2.18	
LYM x10^9/l	<5 years	19	2.7± .73	.127
	5-10 years	8	2.2± .54	
	>10 years	8	2.3± .58	
MID x10^9/l	<5 years	19	.5± .19	.056
	5-10 years	8	.6± .10	
	>10 years	8	.7± .22	
GRA x10^9/l	<5 years	19	3.1± 1.37	.396
	5-10 years	8	3.3± 1.34	
	>10 years	8	3.9± 1.76	
RBC x10^9/l	<5 years	19	5.5± 1.05	.963
	5-10 years	8	5.4± .54	
	>10 years	8	5.4± .49	
HB(g/dl)	<5 years	19	14.8± 1.57	.771
	5-10 years	8	15.1± 1.08	
	>10 years	8	14.5± 2.00	
HCT(%)	<5 years	19	46.6± 7.34	.523
	5-10 years	8	46.2± 4.46	
	>10 years	8	43.6± 5.28	
MCV(fl)	<5 years	19	84.8± 4.69	.984
	5-10 years	8	84.9± 5.08	
	>10 years	8	84.4± 9.46	

MCH(pg)	<5 years	19	27.4± 4.10	.897
	5-10 years	8	27.8± 2.23	
	>10 years	8	26.9± 3.60	
MCHC(g/dl)	<5 years	19	32.2± 4.19	.799
	5-10 years	8	32.6± 1.59	
	>10 years	8	33.1± .74	
RDW(%)	<5 years	19	15.8± 1.03	.346
	5-10 years	8	15.8± .99	
	>10 years	8	16.5± 1.77	
PLT x10^9/l	<5 years	19	308.2± 119.59	.352
	5-10 years	8	292.6± 75.91	
	>10 years	8	245.9± 61.98	
PCT(%)	<5 years	19	.3± .12	.575
	5-10 years	8	.2± .07	
	>10 years	8	.2± .05	
MPV(fl)	<5 years	19	8.5± .76	.143
	5-10 years	8	8.2± 1.34	
	>10 years	8	9.1± .93	
PDW(%)	<5 years	19	39.8± 1.57	.346
	5-10 years	8	42.6± 8.73	
	>10 years	8	40.9± 2.29	

Significance level ($P \leq 0.05$)

Table 3.5: Effect of toombak dipping on complete blood counts according to the number of dipping per day.

Components	Numbers of dipping / day	N	Mean±SD	P. Value
WBC x10^9/l	<15	18	6.3±2.00	.756
	16-30	11	6.9±1.73	
	31-45	3	6.4±1.54	
	>46	3	5.6±1.75	
LYM x10^9/l	<15	18	2.5±.67	.599
	16-30	11	2.7±.75	
	31-45	3	2.7±.70	
	>46	3	2.1±.60	
MID x10^9/l	<15	18	.6±.20	.992
	16-30	11	.6±.16	
	31-45	3	.6±.36	
	>46	3	.6±.16	
GRA x10^9/l	<15	18	3.3±1.54	.881
	16-30	11	3.6±1.42	
	31-45	3	3.1±1.73	
	>46	3	2.9±1.41	
RBC x10^9/l	<15	18	5.5±1.06	.872
	16-30	11	5.6±.62	
	31-45	3	5.3±.13	
	>46	3	5.1±.25	
HB(g/dl)	<15	18	14.5±1.64	.540
	16-30	11	15.2±1.69	
	31-45	3	15.4±.96	
	>46	3	14.3±.53	

HCT(%)	<15	18	45.8±7.98	.852
	16-30	11	46.7±4.68	
	31-45	3	46.0±3.12	
	>46	3	43.0±1.23	
MCV(fl)	<15	18	85.3±5.55	.772
	16-30	11	83.5±7.01	
	31-45	3	87.0±6.00	
	>46	3	83.3±6.11	
MCH(pg)	<15	18	27.1±4.32	.824
	16-30	11	27.3±2.87	
	31-45	3	29.2±1.93	
	>46	3	27.8±2.31	
MCHC(g/dl)	<15	18	32.1±4.25	.874
	16-30	11	32.8±1.66	
	31-45	3	33.3±.36	
	>46	3	33.2±.60	
RDW(%)	<15	18	16.1±1.52	.908
	16-30	11	15.8±1.01	
	31-45	3	16.1±.67	
	>46	3	15.7±.32	
PLT x10^9/l	<15	18	303.6±109.31	.669
	16-30	11	294.5±107.22	
	31-45	3	242.3±69.62	
	>46	3	244.0±32.05	
PCT(%)	<15	18	.2±.10	.752

	16-30	11	.3±.10	
	31-45	3	.2±.06	
	>46	3	.2±.04	
MPV(fl)	<15	18	8.4±.93	.739
	16-30	11	8.8±1.09	
	31-45	3	8.7±.44	
	>46	3	8.5±1.50	
PDW(%)	<15	18	41.0±5.91	.889
	16-30	11	40.0±2.14	
	31-45	3	40.1±1.21	
	>46	3	42.1±.72	

Significance level ($P \leq 0.05$)

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 Discussion:

This study aimed to measure the complete blood count of the toombak dipping males in Khartoum state, because of the scarcity of data that support the association between toombak dipping and the change of dippers CBC, these results were taken from of other types of tobacco either smoking or smokeless.

TWBCs showed significant increase in toombak dippers in comparison of non-toombak dippers this agrees with the previous studies of smokeless tobacco (Rajasekhar, *et.al.*, 2007 , Jaganmohan and Sarma, 2011 , Mukherjee and Chatterjee, 2013 and Kumar, *et.al*., 2017), and studies of smoking tobacco in (Kurtoğlu, *et.al*., 2013 , Nadia, *et.al*., 2015 , Bashir, *et.al.*, 2016 , Mustafa, 2017 and Malenica, *et.al*., 2017 , Soflaei, *et.al*., 2018), Some authors claim that the increase in the number of leukocytes can be the consequence of nicotine induced release of catecholamine and steroid hormones from the core of the adrenal gland. It is known that an increase in the level of certain endogenic hormones, such as epinephrine and cortisol, cause an increase in the number of leukocytes (Kapoor and Jones, 2005 and Deutsch, *et.al*., 2007). Additionally, the increase of WBCS count may be a result of the chronic ulceration in the gum or the lips which are the site of dipping in the mouth.

The RBCs count showed significant increase which accords with the result of chewing tobacco (gutkha) in India (Mukherjee and Chatterjee, 2013 and Kumar, *et.al*., 2017) and in cigarette smoking studies of (Nadia, *et.al*., 2015 , Soflaei, *et.al*., 2018 , Mustafa, 2017 and Bashier, *et.al.*, 2016) and in studies of Shisha in (Nadia, *et.al*., 2015 and Soflaei, *et.al*., 2018). The elevated RBCs count can lead to polycythemia which slow blood velocity and increase the risk of intravascular clotting. The increased total erythrocyte count of gutkha users seems to reflect that consuming gutkha may also stimulate erythropoiesis. Mice treated with gutkha showed insignificant rise in total

erythrocyte count, insufficient pulmonary function in gutkha consumers may impart a necessity of stimulating erythropoiesis for fulfilling the oxygen demands of the body (Shahla , 2007).

Hematocrit was significant raised due to toombak dipping this is on line with the previous studies of smokeless tobacco in Turkey (Guven and Tolun, 2012) and in India (Mukherjee and Chatterjee, 2013), and in studies of smoking cigarette by (Nadia, *et.al*., 2015 / Bashier, *et.al.*, 2016 / Mustafa, 2017 and Soflaei, *et.al*., 2018) , Elevated levels HCT 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 is a significant reduction in MCH and MCHC which may indicate the presence of anisopoikilocytois (Ghosh, *et.al.,* 2012).

RDW is significantly increased which is corresponding with the studies on cigarette smoking done by (Kurtoglu, *et.al*., 2013 / Mustafa, 2017 and Soflaei *et.al.,* 2018) and Shisha study of (Soflaei *et al* 2018). RDW is widely used as a guide for the differential diagnosis of anemia, with high values found in increased RBC destruction (hemolytic anemias) or defective erythropoiesis for example, nutritional deficiencies of iron, folic acid, and vitamin B_{12}, or blood transfusion. It has been shown that RDW is elevated in cardiovascular and pulmonary diseases. Increased RDW has also been noted in a variety of non cardiovascular disease states including liver disease, inflammatory bowel disease, occult colon cancer and neoplastic metastases to the bone marrow. (kurtoglu, *et.al*., 2013). High RDW may serve as an important biomarker in a variety of acute and chronic pathological conditions (Dogan, *et.al.*, 2015)

The elevated platelet count found in this work agrees with a study of smokeless tobacco (Maras) in Turkey (Guven and Tolun, 2012). Nicotine has been reported to injure endothelial cells in animal studies (Thyberg, 1986 and Krupski, 1987). Nicotine has been found to release growth factors and to promote angiogenesis, which could contribute to atherogenesis (Heeschen, *et.al*, 2003). The endothelium plays a central role in the modulation of vascular tone, the inhibition of platelet aggregation and vascular smooth muscle proliferation. Platelet play a pivotal role in atherothrombosis, the major cause of most unstable coronary syndromes (Davi and Patrono, 2007).

Mean platelet volume (MPV), the most commonly used measures of platelet size, is a potential marker of platelet reactivity (Kamath, 2001).there is a significant decrease in MPV in this study, low MPV characterizes a reactive thrombocytosis seen in infection, inflation and malignancy.(Chu, *et.at*, 2010).

The mean monthly cost of toombak dipping is approximately 230 SDG which is high if compared to the minimum wage in Sudan which is 425.

4.2 Conclusion:

-Toombak dipping has effect on CBC parameters (raised the mean values of the WBCS, RBCS, PCV, RDW and PLT), (lowered the mean values of the

MCH, MCHC and MPV) and no effect on mean values of (LYM, MID, GRA, HGB, MCV, PCT and PDW).

-Age of toombak dippers show a variations on the values of LYM.

-Duration of toombak dipping and the numbers of toombak dipping / day has no effect on CBC.

4.3 Recommendation:

Since toombak dipping is widespread in Sudan, it is recommended that:

-Increase public awareness of the potential health hazards of toombak dipping.

-Support scientific research on toombak dipping in different institutions and universities to explore the different effects of toombak dipping on public health.

-Further studies should be done to assess the effect of toombak dipping on blood film, PLT activity, coagulation profile, thrombopoietin hormones and cyclooxygenase enzymes.

-cross random studies are needed to cover other states in Sudan.

-Studies of behavioral counseling sustained release bupropion hydrochloride therapy and nicotine replacement therapy may be safe therapeutic modalities for treatment of smokeless tobacco use.

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Appendix–1

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of graduate Sciences

RESEARCH QUESTIONNAIRE

Serial No: ……..

Age: <20 ☐ 21-40 ☐ 41-60 ☐ >60 ☐

Marital status: married ☐ single ☐

Education: illiterate ☐ primary ☐ secondary ☐ higher ☐ education

Occupation: student ☐ driver ☐ teacher ☐ medical field ☐ Other ☐……

For how long have you been dipping?

<1Year ☐ 1-5 Years ☐ 5-10 Years ☐ >10 Years ☐

How many times do you dip daily?

5-15 ☐ 16-25 ☐ 26-35 ☐ >36 ☐

Site of dipping: Buccal ☐ Lower lip ☐ upper lip ☐ mixed ☐

How match you spend daily on dipping?

……..

What are the motivating factors that drive you to dipping?

……

……

……

Does the dipping affect the appetite? Yes ☐ No ☐

Do you feel any nausea or dizziness when dipping? Yes ☐ No ☐

Do you get any shiver when dipping? Yes ☐ No ☐

Do you have any acute or chronic diseases in your respiratory system?

Yes ☐ No ☐

Do you have any acute or chronic diseases in your digestive system?

Yes ☐ No ☐

Do you have any health problems in your mouth? Yes ☐ No ☐

If yes where:

Tongue: change color ☐ sores ☐

Teeth: change color ☐ toothache ☐ tooth decay ☐

Gums: gingivitis ☐ ulceration ☐ discoloration ☐

Lips: crack ☐ ulceration ☐ dryness ☐

Signature: ……

Appendex-2

Fig (1) nicotiana rustica

Fig(2) Diagram of Dry Sunff

Fig (3) Diagramof Moist Sunff

Fig (4) Diagram of addition of natron to dry Sunff

Appendex-3

Hemolyzer® 3 Pro

analyticon

A step further in 3-part differential hematology