بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Reference Values of Blood Count among Healthy Adult Sudanese Males

القيم المرجعية لتعداد خلايا الدم في الذكور البالغين الاصحاء السودانيين

A dissertation Submitted in Partial Fulfillment for Requirements of MSc Degree in Medical Laboratory Science (Hematology and Immunohematology)

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بسم الله الرحمن الرحيم

يقول الله تعالى :

{ لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِين }

سورة البقرة الآية رقم (٢٨٦)

Dedication

To …

The reason of what I become today. Thanks for your great support and continuouscare, (My mother).

To …

Who I hold his name will all honor, (My father)

I am really grateful to both of you. You have been my inspiration and my soul mates, (My brothers)

To …

My life flavors, (My wife and my son)

To ..

The absolutely necessary person. The gentle reader.

Abo Moumin

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Thanks for my god **Allah** firstly and lastly, enabled me to conduct this study by his blessing therefore.

Great thanks for **my family** which always encourage me for the better.

Thanks for **my friends**, who helped me and made me going on.

Thanks for **my supervisor,** who guide me on this research**.**

Last thanks for **everyone** who helped me in my research.

ABSTRACT

Descriptive cross-sectional study was conducted to determine the normal reference values of hemoglobin, Hematocrit, red blood cells, red blood cell indices, white cells and platelets counts in adult healthy Sudanese male. Total numbers of samples 625 healthy individuals from most common tribes in Sudan.3ml of EDTA anti coagulated venous blood sample were collected from each subject. The complete blood count (CBC) was measured by using automated hematological analyzer (Sysmex kx-21N). Analysis was done in ALAMAL National Hospital. The ages of the subjects ranged from 18 to 68 years. A questionnaire and informed consent was filled by each subject. The results showed that the Hb (14.6±1.48)g/dl.HCT (44.9±4.9) %. RBCs count (5.3±0.59)× 10^6/μL. MCV (84.4±6.4) fl. MCH (27.5 ±2.1) pg. MCHC (32.7±2.3)g/dl. WBCs count (6.6±1.96) × 10^3/μL. Platelet count (272.6±66.3) ×10^3/μL.

المستخلص

أجريت دراسة وصفية مقطعية لتحديد القيم المرجعية الطبيعية لخضاب الدم، حجم الخلايا المعبأة، خلايا الدم الحمراء، مؤشرات خلايا الدم الحمراء، والخلايا البيضاء والصفائح الدموية في الذكورالاصحاء البالغين السودانيين. إجمالي عدد العينات ٦٢٥ عينه من الأشخاص الأصحاء من معظم القبائل المعروفة في السودان. تم جمع ٣ملم من الدم الوريدي في مادة EDTA المضادة للتخثر من كل موضوع. تم قياس تعداد الدم الكامل (CBC) باستخدام محلل الدم الآلي (Sysmex KX-21N) . وقد تم التحليل في مستشفى الامل الوطني. كانت تتراوح أعمارالمشاركين من ١٨الي٦٨ عاما. وقد تم ملأ استبيان واقرار مشاركة من قبل كل مشارك. اظهرت النتائج ان خضاب الدم (1.48±14.6) جم/دل . حجم الخلايا المعبأة (4.9±44.9)%.عدد كريات الدم الحمراء (0.59±5.3) × $١٠^{٦}$/ ميكرو ليتر.متوسط حجم الخلية(6.4±84.4)fl. متوسط سعة الخلية من خضاب الدم (2.1±27.5)pg. متوسط تركيز خضاب الدم في الخلية (2.3±32.7)جم/دل.عدد الكريات البيضاءهو (1.9±66.6) × $١٠^{٣}$/ ميكروليتر.الصفيحات الدمويه (66.3±272.6) × ١٠/ ميكروليتر.

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List of Abbreviations

NR	Normal reference values
RBCs	Red blood cells
Hb	Hemoglobin
HCT	Hematocrit
MCV	Mean cell volume
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
WBCs	White cells
PLT	Platelets counts
CBC	Complete blood count

Chapter One

Introduction and Literature Review

Chapter One

Introduction and Literature Review

1.1 Introduction

A reference range is usually defined as the set of values 95 percent of the normal population falls within (that is, 95% prediction interval). It is determined by collecting data from vast numbers of laboratory tests. A number of factors affect hematological values in apparently healthy individuals. These include the technique and timing of blood collection, transport and storage of specimens, differences in the subject's posture when the sample is taken, prior physical activity, or whether the subject is confined to bed. Variation in the analytic methods used may also affect the measurements. These can all be standardized (weed, 1963).

More problematic are the inherent variables as a result of sex, age, occupation, body build, genetic background, and adaptation to diet and to environment (especially altitude). These factors must be recognized when establishing physiologically normal values. Furthermore, it is difficult to be certain in any survey of a population for the purposes of obtaining data from which normal ranges may be constructed that the “normal” subjects are completely healthy and do not have nutritional deficiencies (especially iron deficiency, which is prevalent in many countries), mild chronic infections, parasitic infestations, or the effects of smoking. Hematological values for the normal and abnormal will overlap, and a value within the recognized normal range may be definitely pathological in a particular subject. For these reasons the concept of “normal values” and “normal ranges” has been replaced by reference values and the reference range, which is defined by reference limits and obtained from measurements on the reference population for a particular test. The reference range is also termed the reference interval. Ideally, each laboratory should establish a databank of reference values that take account of the variables mentioned earlier, so that an individual's result can be expressed and interpreted relative to a comparable apparently normal population, insofar as normal can be defined (Lewis, 2006).

Health and disease can only be distinguished by accurate and reliable reference values of a particular laboratory test. It is now a proven fact that there is considerable variation in hematology reference intervals depending on the demographic and preanalytical variables. There are evidences that values provided by manufacturers do not have appropriate application for all populations. Moreover, reference ranges provided by different laboratory manuals and books also do not solve this problem (Bain, 2002).

In daily practice in Sudan, it is important to have hematological reference values for diagnosis orientation and treatment decision. The hematological reference values were determined many years ago for the Caucasian's populations. Analysis by different statistical techniquesand variations with age and sex. Recently, several authors tried to establish reference values in hematology for African countries (Lewis, 2006).

However there are some discrepancies from one study to another which may be related to different factors such as age, sex, geographic origin, altitude, and ethnic origin (Lugada, 2004).

Moreover, many constitutional hemoglobin abnormalities (thalassemia, sickle cell disease and hemoglobin C) or pathologic conditions (malaria, HIV, HBV and HCV viral infections), influence the hematological values (Saathoff, 2008).

1.2 Literature Review

A complete blood count (CBC), also known full blood count (FBC), or full blood exam (FBE), is a blood panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood, such as the cell count for each cell type and the concentrations of various proteins and minerals. A scientist or lab technician performs the requested testing and provides the requesting medical professional with the results of the CBC.

The cells that circulate in the bloodstream are generally divided into three types: white blood cells (leukocytes), red blood cells (erythrocytes), and platelets (thrombocytes). Abnormally high or low counts may indicate the presence of many forms of disease, and hence blood counts are amongst the most commonly performed blood tests in medicine, as they can provide an overview of a patient's general health status. A CBC is routinely performed during annual physical examinations in some jurisdictions Procedure. A phlebotomist collects the sample through venipuncture, drawing the blood into a test tube containing an anticoagulant (EDTA, sometimes citrate) to stop it from clotting. The sample is then transported to a laboratory. Sometimes the sample is drawn off a finger prick using a Pasteur pipette for immediate processing by an automated counter. A complete blood count (CBC) is a blood test used to evaluate your overall health and detect a wide range of disorders, including anemia, infection and leukemia.

A complete blood count test measures several components and features of your blood, including:

- Red blood cells, which carry oxygen.
- Hemoglobin, the oxygen-carrying protein in red blood cells.

- Hematocrit, the proportion of red blood cells to the fluid component, of plasma, in your blood.
- Red cell indices which can help in differentiation what type of anemia.
- White blood cells, which fight infection.
- Platelets, which help with blood clotting. (Gomella, 2013).

1.2.1 Hemoglobin

Also spelled haemoglobin and abbreviated Hb or Hgb, is the iron-containing oxygen-transport metallo protein in the red blood cells of all vertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues). There it releases the oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism (Maton, 1993).

Hemoglobin has an oxygen-binding capacity of 1.34 mL O2 per gram (Dominguez, 1981).

This increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules (Costanzo, 2007).

Hemoglobin is involved in the transport of other gases. It carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen (Epistien,1998).

Hemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain hemoglobin include the A9 dopaminergic neurons in the substantianigra, macrophages, alveolar cells, and mesangial cells in the kidney. In these tissues, hemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism.

Hemoglobin and hemoglobin-like molecules are also found in many invertebrates, fungi, and plants. In these organisms, hemoglobin's may carry oxygen, or they may act to transport and regulate other things such as carbon dioxide, nitric oxide, hydrogen sulfide and sulfide. A variant of the molecule, called leghemoglobin, is used to scavenge oxygen away from anaerobic systems, such as the nitrogen-fixing nodules of leguminous plants, before the oxygen can poison the system (Biagilio, 2009).

1.2.2 Hematocrit

Hematocrit is a blood test that measures the percentage of the volume of whole blood that is made up of red blood cells. This measurement depends on the number of red blood cells and the size of red blood cells. The hematocrit is almost always ordered as part of a complete blood count (CBC).This testmay orderto investigate anemia, diet deficiency, leukemia and other medical condition (Biagilio, 2009).

1.2.3 Red blood cell

Erythropoiesis is the development process by which new erythrocytes are produced, it lasts about 7 days. Through this process erythrocytes are continuously produced in the red bone marrow of large bones, at a rate of about 2 million per second in a healthy adult. (In the embryo, the liver is the main site of red blood cell production.) The production can be stimulated by the hormone erythropoietin (EPO), synthesized by the kidney. Just before and after leaving the bone marrow, the developing cells are known as reticulocytes, these comprise about 1% of circulating red blood cells.

Red blood cells (RBCs), also called erythrocytes, are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O_2) to the body tissues via blood flow through the circulatory system. RBCs take up oxygen in the lungs or gills and release it into tissues while squeezing through the body's capillaries. The cytoplasm of erythrocytes is rich in hemoglobin, an iron-containing biomolecule that can bind oxygen and is responsible for the red color of the cells. The cell membrane is composed of proteins and lipids, and this structure provides properties essential for physiological cell function such as deformability and stability while traversing the circulatory system and specifically the capillary network. In humans, mature red blood cells are flexible and oval biconcave disks. They lack a cell nucleus and most organelles, in order to accommodate maximum space for hemoglobin. Approximately 2.4 million new erythrocytes are produced per second in human adults, quarter of the cells in the human body are red blood cells. Red blood cells are also known as, red cells. Red blood corpuscles (an archaic term), haematids, erythroid cells or erythrocytes (from Greek*erythros* for "red" and *kytos* for "hollow vessel", with -*cyte* translated as "cell" in modern usage).

The functional lifetime of an erythrocyte is about 100–120 days, during which time the erythrocytes are continually moved by the blood flow push (in arteries), pull (in veins) and a combination of the two as they squeeze through micro vessels such as capillaries (Lang, 2008).

Increase in red blood cells known as polycythemia, result from:-

- Dehydration
- Lung (pulmonary) disease
- Kidney or other tumor that produces excess erythropoietin

- Smoking
- Genetic causes (altered oxygen sensing, abnormality in hemoglobin oxygen release).
- Polycythemia Vera a rare disease.

Decrease in red blood cells known as anemia result from:-

- Acute or chronic bleeding
- RBC destruction (e.g., hemolytic anemia, etc.)
- Nutritional deficiency (e.g., iron deficiency, vitamin B12 or folate deficiency)
- Bone marrow disorders or damage
- Chronic inflammatory disease
- Kidney failure

1.2.4 Red cell indices

1.2.4.1 MCV

Decrease Mean Corpuscular Volume Indicates RBCs are smaller than normal (microcytic), caused by iron deficiency anemia or thalassemias, for example. Increase Indicates RBCs are larger than normal (macrocytic), for example in anemia caused by vitamin B12 or folate deficiency.

1.2.4.2 MCH

Mean Corpuscular Hemoglobin Mirrors MCV results; small red cells would have a lower value. Mirrors MCV results, macrocytic RBCs are large so tend to have a higher MCH.

1.2.4.3 MCHC

Mean Corpuscular Hemoglobin Concentration may be low when MCV is low; decreased MCHC values (hypochromia) are seen in conditions such as iron deficiency anemia and thalassemia. 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.

1.2.5 White blood cells (WBCs)

Also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All leukocytes are produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system (Maton, 1993).

Five different and diverse types of leukocytes exist. These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocytic (La Fleur, 2008).

The number of leukocytes in the blood is often an indicator of disease, and thus the WBC count is an important subset of the complete blood count. The normal white cell count is usually between 4 and 11×10^9/L. In the US this is usually expressed as 4,000–11,000 white blood cells per microliter of blood. They make up approximately 1% of the total blood volume in a healthy adult.An increase in the number of leukocytes over the upper limits is called leukocytosis result from:-

- Infection, most commonly bacterial or viral
- Inflammation

- Leukemia, myeloproliferative disorders
- Allergies, asthma
- Tissue death (trauma, burns, heart attack)

And a decrease below the lower limit is called leucopenia, result from:-

- Bone marrow disorders or damage
- Autoimmune conditions
- Severe infections (sepsis)
- Lymphoma or other cancer that spread to the bone marrow
- Diseases of immune.

The name "white blood cell" derives from the physical appearance of a blood sample after centrifugation. White cells are found in the buff, a thin, typically white layer of nucleated cells between the sedimented red blood cells and the blood plasma. The scientific term leukocyte directly reflects its description. It is derived from the Greek word leuko- meaning "white" and kytos meaning "hollow vessel", with -cyte translated as "cell" in modern use. The buffy coat may sometimes be green if there are large amounts of neutrophils in the sample, due to the hem-containing enzyme myeloperoxidase that they produce (Bruce,2002).

Types:-

1.2.5.1 Neutrophil

Neutrophils are the most abundant white blood cell, constituting 60-70% of the circulating leukocytes (Bruce, 2002).

They defend against bacterial or fungal infection. They are usually first responders to microbial infection; their activity and death in large numbers forms pus. They are commonly referred to as polymorph nuclear (PMN) leukocytes, although, in the technical sense, PMN refers to all granulocytes. They have a multi-lobed nucleus, which consists of three to five lobes connected by slender strands (Salandin, 2012).

This gives the neutrophils the appearance of having multiple nuclei, hence the name polymorph nuclear leukocyte. The cytoplasm may look transparent because of fine granules that are pale lilac when stained. Neutrophils are active in phagocytizing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew their lysosomes (used in digesting microbes) and die after having phagocytized a few pathogens (Wheater, 2002).

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, 2010).

1.2.5.2 Eosinophil

Eosinophil's 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. Eosinophil's 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 that are too big for any one WBC to phagocytize. In general, their nucleus is bi-lobed. The lobes are connected by a thin strand. The cytoplasm is full of granules that assume a characteristic pink-orange color with eosin staining (Pillay, 2010).

1.2.5.3 Basophil

Basophiles are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing the dilation of blood vessels. Because they are the rarest of the white blood cells (less than 0.5% of the total count) and share physicochemical properties with other blood cells, they are difficult to study. They can be recognized by several coarse, dark violet granules, giving them a blue hue. The nucleus is bi- or tri-lobed, but it is hard to see because of the number of coarse granules that hide it.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. Basophils can also release chemical signals that attract eosinophil’s and neutrophils to an infection site (Pillay, 2010).

1.2.5.4 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:

1.2.5.4.1 B cells

Make antibodies that can bind to pathogens, block pathogen invasion, activate the complement system, and enhance pathogen destruction.

1.2.5.4.2 Tcells

CD4+ helper T cells: T cells displaying co-receptor CD4 are known as CD4+ T cells. These cells have T-cell receptors and CD4 molecules that, in combination, bind antigenic peptides presented on major histocompatibility complex (MHC) class II molecules on antigen-presenting cells. Helper T cells make cytokines and perform other functions that help coordinate the immune response. In HIV infection, these T cells are the main index to identify the individual's immune system integrity.CD8+ cytotoxic T cells.T cells displaying co-receptor CD8 are known as CD8+ T cells. Nearly all nucleated cells display MHCI.γδ T cells possess an alternative T cell receptor (different from the αβ TCR found on conventional CD4+ and CD8+ T cells). Found in tissue more commonly than in blood, γδ T cells share characteristics of helper T cells, cytotoxic T cells, and natural killer cells. Natural killer cells are able to kill cells of the body that do not display MHC class I molecules, or display stress markers such as MHC class I polypeptide-related sequence A (MIC-A). Decreased expression of MHC class I and up-regulation of MIC-A can happen when cells are infected by a virus or become cancerous (Kenneth, 2007).

1.2.5.5 Monocyte

Monocytes, the largest type of WBCs, share the "vacuum cleaner" (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 attacking 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. They have the kidney shaped nucleus and are typically a granulated. They also possess abundant cytoplasm.Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and are then known as macrophages (Kenneth, 2007).

1.2.6 Blood Platelet

Also called thrombocytes, are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clogging blood vessel injury.

Platelets have no cell nucleus, they are fragments of cytoplasm which are derived from the megakaryocytes of the bone marrow, and then enter the circulation. These inactivated platelets are biconvex discoid (lens-shaped) structures 2–3 µm in greatest diameter.Platelets are found only in mammals, whereas in other animals (e.g. birds, amphibians) thrombocytes circulate as intact mononuclear cells. On a stained blood smear, platelets appear as dark purple spots, about 20% the diameter of red blood cells.The main function of platelets is to contribute to hemostasis, disorder of platelet function is a thrombocytopathy .Low platelet concentration is thrombocytopenia and is due to either decreased production or increased destruction.

Examples for causes of thrombocytopenia:-

- Viral infection (mononucleosis, measles, hepatitis).
- Rocky mountain spotted fever
- Platelet autoantibody

- Drugs (acetaminophen, quinidine, sulfa drugs).
- Cirrhosis
- Autoimmune disorders.
- Sepsis .
- Leukemia, lymphoma.
- Myelodysplasia.
- Chemo or radiation therapy.

Elevated platelet concentration is thrombocytosis and is either congenital, reactive (to cytokines), or due to unregulated production one of the myeloprolerativeneoplasm's or certain other myeloid neoplasm's.

Examples for causes of thrombocytosis:-

- Cancer (lung, gastrointestinal, breast, ovarian, lymphoma).
- Rheumatoid arthritis, inflammatory bowel disease, lupus.
- Iron deficiency anemia.
- Hemolytic anemia.
- Myeloproliferative disorder as essential thrombocythemia (Laki, 1972).

1.3 Rationale

Health and disease can only be distinguished by accurate and reliable reference values of a particular laboratory test. It is now a proven fact that there is considerable variation in hematology reference intervals depending on the demographic and pre analytical variables (Saathoff, 2008).

Sudan is a North African country, with an Altitude/ elevation 380 m (1247 ft). According to study done in 2008 Sudan population will be in 2015 about (38,435,252) individuals. Males in ages between 18-68 about (8,725,912) male (population projection Sudan, 2008).

Until now, blood cells and red cells indices reference values have not been established. The values usually used are those of Caucasian populations. According to this context and for a better interpretation of hematologic results, so it necessary to establish adult blood cells and red cells indices reference values in Sudan. Till now Sudanese physicians and doctors depend on the universal reference values of hemoglobin, RBCs, WBCs and PLTs in diagnosis of diseases. Few studies were done in this filed in specific population not representing all Sudanese population. Approximately most of Sudanese trips included in this study to generalize and justify the results. In daily practice in Sudan, it is important to have hematological reference values for diagnosis orientation.

1.4 Objectives

1.4.1 General Objective

To determine the normal hematological parameter in Sudanese adult males.

1.4.2 Specific Objectives

.To establishes reference values for hemoglobin concentration and Hematocrit.

.To establishes reference values for Red blood cells count and indices.

.To establishes reference values for White blood cells and Platelets count.

Chapter Two

Materials and Methods

Chapter Two

Materials and Methods

2.1 Study design and time

Descriptive cross-sectional study, conducted during the period of March 2015 to October 2015.

2.2 Study area

The study was conducted in Khartoum state.

2.3 Study Population

Apparently healthy none hospitalized adult Sudanese males.

2.4 Inclusion criteria

Healthy Sudanese male's ages ranged 18 – 68 year.

2.5 Exclusion criteria

* Those whom complaining from any infection or disease.

* Those individual under 18 years old and above 68 years old.

* Smokers males.

2.6 Sample size and procedure

625 sample .Samples were collected by using dry, vacuum system, tourniquet was used to make the veins more prominent, blood samples (3ml) was collected in EDTA vacuum tube from each volunteer under septic condition.

2.6.1 Material

1- EDTA container.

2- Disposable syringes.

3- Cotton wool.

4- Sysmex Kx.21N.

2.6.2 Reagent

1- Cell pack (diluents).

2- Stromatolyser .

3- Cell clean.

2.6.3 Procedure

The reagent for operation were checked ,then the power switch was turn on auto rinse and back ground check were automatically performed then three level of control (low count, normal count and high) were applied after selection whole blood mode of analysis sample number were introduced by pressing sample number key then enter key was pressed after that sample was mixed carefully the tab bring in close contact with sample probe and the start key was pressed, after the required volume of blood were aspirated ,then tube was removed ,result was displayed the screen and printout.

2.6.4 Quality control of Sysmex

Hematological analyzer provide quick and accurate results in most situation, however, false results related either to platelets or other parameter from complete blood cells count may be observed in several instances, falls low WBCs count may be observed because of agglutination in the presence of EDTA(Seysmex, American ,2003)

The sample size calculated with known population size as follow.

$$n = \frac{?}{1+N(d)^2}$$

n=sample size.

N= population size.

d= degree of precision (0.04).

Sample size = 8725912/1+8725912(0.04) ^{2} =**625**

2.7 Ethical Considerations

A Study was approved from ethical committee of the Sudan University of Science and Technology, verbal informed consent was obtained and all patients were informed by aims of the study.

2.8 Data collection

Data were collected using structural interviewing questionnaire, which was designed to collect and maintain all information needed.

2.9 statistical analyses

Data were analyzed by SPSS statistical package of social science (version 11.5, SPSS Inc).

Chapter Three

Results

Chapter Three

Results

3. Results

In this study 625 subjects were chosen for determination of normal values for Hb RBCs, RBCs indices, total WBCs, and platelets count in healthy adult Sudanese males during the period march to October 2015.

49 different Sudanese tribes were included in this study, the table below show the highest frequency of tribes in study group.

Table (3.1) highest frequency of tribes in study group

tribe	frequency
Galey	١٢٠
Shaigey	٨١
Donglawey	٥٤
Kahley	٣٨
Hahasy	٣٧
Bedarey	٣٣
Flaty	٢٨
Hlfawey	٢١
Hsaney	١٧
Zgawey	١٥

Young age's groups were found healthier than the elder one. So the lowers frequencies were elder age's groups.

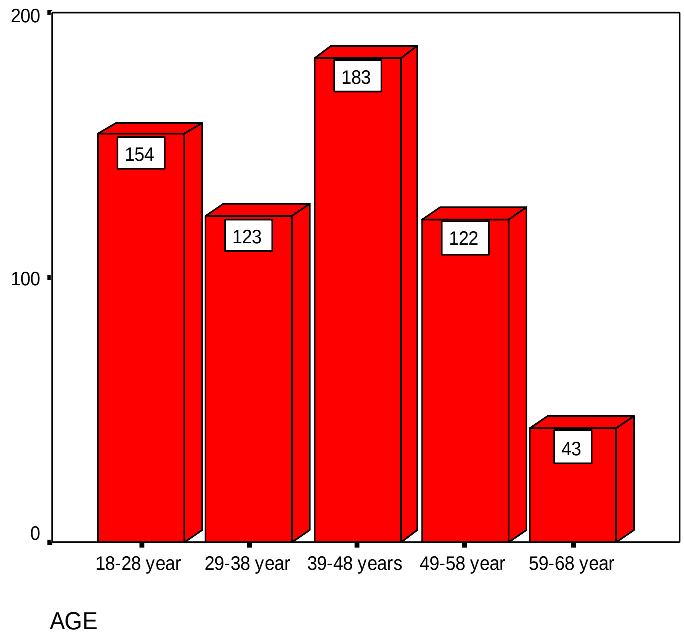

Fig (3.1) frequency of tested group according to age

The highest frequency is group of age 39-48 and the lower frequency was group of age 59-68

Table (3.2) complete blood count parameters of study group

Parameter	Mean±standard deviation
HGB g/dl.	14.6 ± 1.48
HCT %.	44.9 ± 4.9
RBC $\times 10^6/\mu L$.	5.3 ± 0.59
MCV fl.	84.4 ± 6.4
MCH pg.	27.5 ± 2.1
MCHC g/dl.	32.7 ± 2.3
WBC $\times 10^3/\mu L$.	6.6 ± 1.96
PLT $\times 10^3/\mu L$.	272.6 ± 66.3

Chapter Four

Discussion, Conclusion and Recommendations

Chapter Four

Discussion, Conclusion and Recommendations

4.1 DISCUSSION

Sudan forms one of the largest part of Africa occupies over 1.0 million square kilometers with an Altitude/ elevation 380 m (1247 ft). According to study done in 2008 Sudan population will be in 2015 about (38,435,252) individuals.

It is important to have hematological reference values for diagnosis orientation. For this study, we investigated adult Sudanese male in age between 18 and 68 year for determination of normal values for Hb, RBCs count RBCs indices, total WBCs, and platelets. Approximately most of Sudanese trips included in this study to generalize and justify the results

Few studies were done in this filed in specific population not representing all Sudanese population, one of these studies was done in Khartoum north area in Sudan with sample size (200 male), the results are relatively close to results that obtained in this study (Osman , 2013).

Hemoglobin in male of Sudanese population (14.7±1.5)g/dl is less than hemoglobin in male British population (15.7±2.25) g/dl. But higher than Normal Hb in male of Saudis population (14.2±1.1)g/dl.(Alhazmi, 2001).

Normal RBCs Count in male of Sudanese population $(5.3\pm0.6)\times10^6$/ μL is slightly increase than male of Saudis population $(5.1\pm0.26)\times10^6$/ μLand than male of British population $(5.0\pm0.5)\times10^6$/ μL. But less than American population in the upper limit Normal Values males (4.5 to 6.0) million/cu mm blood.

German population and American population are the similar to each other in red count for both sexes.

The normal TWBCs count of Sudanese population $(6.6 \pm 1.96)\times10^3/\mu L$ is higher than Black population of the South Africa$(5.60 \pm 1.51)\times10^3/\mu L$. But less than males of British population $(7.0\pm3.0)\times10^3/\mu L$, and males of Saudis population $(7.9\pm2.1)\times10^3/ \mu L$ (Badenhorst, 1995).

In previous studies in Britain (that a different ethnic living in the same community in Britain Total and differential white cell counts were studied in 399 women living in the same community in Britain but drawn from four different ethnic groups. The groups were white (northern European), Indian, black (African and West Indian), and Oriental. The total white cell count and absolute neutrophil count were significantly lower in the black group than in each of the other groups. The differences between white cell counts of Indians and Orientals and those of whites are minor and for practical purposes they can be assessed in relation to reference range derived for white s) (Bain, 1984).

This research confirms that the genes are related to variation of white count cells.

Platelet count in Sudanese males $(272.7\pm66)\times10^9/l$ is slightly less than Black males of the Witwatersrand (South Africa) Platelet counts $(280\pm59.4)\times10^9/l$ (Badenhorst, 1995). But higher than males of Togo $236\times10^9/L$. (Hindawi.com)

Platelet count in American population $150{,}000\text{-}400{,}000/mm^3$.And British population 150,000 to 450,000 platelets per micro liter of blood are similar to each other ,but higher and wider in the normal range if compared with Sudanese.

4.2 CONCLUSION

- Reference values of hemoglobin in Sudanese adult males are higher than hemoglobin in Saudis adult male, But less than hemoglobin in males of British.
- Reference values of RBCs Count in Sudanese adult male population less than American population in the upper limit Normal Values.
- Reference values of TWBCs count of Sudanese adult males is higher than Black males of the South Africa.
- Reference values of Platelet count in Sudanese adult males higher than platelets in males of Togo.

4.3 RECOMMENDATIONS

- Reference values of hematological parameter for females and children should be done.
- Other tribes should be investigated for hematological reference values.
- Another study with larger sample size should be done to establish our own reference values of complete blood count parameter.

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Appendices

Appendices (1)

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Date: ………………………………………….   ID NO: …………………………………………..

Name: …………………………………………..   Telephone NO: ………………………………

Address: ………………………………………..   State: ……………………………………….…….

Tribe: …………………………………………….

Age group: 18 – 28 ( )   29 – 38 ( )   39– 48( )   49 – 58( )   59 – 68 ( )

Medical history:-

Did you suffer from any one of these diseases?

	Yes	no
1- Chronic diseases	( )	( )
2- Genetic diseases	( )	( )
3- Hepatitis	( )	( )
4- Syphilis	( )	( )
5- HIV	( )	( )

	Yes	no
Did you undergo to surgical operation during the last 12 month?	( )	( )
Did you donate or receive blood during the last 6 month?	( )	( )
Did you infected with malaria during the last month?	( )	( )
Are you using any type of medication?	( )	( )

If yes mention ……………………………..

Are you smoker?	( )	( )

Clinical examination:-

General appearance ………………………..

Blood pressure ……...............................

Appendices (2)

Principle of Sysmex kx.21N

Sysmex kx.21N the sysmex is hematology automated analyzer use to quickly perform full blood count and reticulocyte count .it is made by the sysmex corporation .Blood is sample and diluted and moves through a tube thin enough that cell pass by one at time, because not everything about the cells can be measured at the same time, blood is separated into number of different channels. As the cells pass through apertures the signals are transmitted in sequence to analog circuit and then to particle size distribution analysis circuits for conversion to cumulative cell size distribution data particle size distribution curves constructed and the auto discrimination level is then set by the microprocessor for each population, This floating threshold allow for discrimination of all population, the cell count include the pulses between the lower and the upper auto discrimination level.

Appendices (3)

بسم الله الرحمن الرحيم

جامعه السودان للعلوم والتكنولوجيا

كليه الدراسات العليا

ماجستير مختبرات طبيه

تخصص علم امراض الدم ومبحث المناعه الدمويه

اقرار مشاركه

الاسم:..

سوف يتم اخذ عينه من الدم (٣مل) من الوريد بواسطة حقنه طعن وذلك بعد مسح منطقة اخذ العينه بواسطة المطهر.

كل الادوات المستخدمة لاخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية.

اوافق انا المزكور اعلاه اخذ عينه لاجراء الدراسة.

الامضاء:

التاريخ:

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