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Determination of Some Hematological Parameters among Healthy Smokers in Khartoum State.

قياس بعض معدلات الدم لدى المدخنين الأصحاء في ولاية الخرطوم

A thesis submitted as partial fulfillment for requirement of Master Degree in Medical Laboratory Science

(Heamatology and Immune Heamatology)

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2015

الآية:

بسم الله الرحمن الرحيم

قال تعالى:

فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ
يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُلْ رَبِّ زِدْنِي عِلْمًا

صدق الله العظيم

سورة طه الآية (114)

Dedication

To…

Our Parents

To…

Our families

Our Lovely Teachers…

Friends (specially the lovely girl enasabdalkhaliq) and all who supported us to finish this work...

We are nothing without your assistances….

ACKNOWLEDGMENTS

Firstly Great Thanks For Compassionate God Allah For his immortal grace, then thanks introduced to our supervisor:D. Munsoor Mohammed Munsoor in hematology department for helping , supporting , vital assistance and advice. Also great thanks to all teachers in hematology department and other teachers of the college….

Abstract

Cigarette Smoking carries higher risks for most of chronic disease, it also has chronic and acute effects on hematological parameters . This descriptive (case – control) analytical study carried out in Khartoum state from April to December 2014 to determine the effect of cigarette smoking on some of blood values on healthy smokers .

In this study cigarette smokers of hundred males participant (50 smokers and 50 non smokers), between the age of 17-60 years old in Khartoum state were investigated , used structural questionnaire and blood sample were taken .

The result of this study revealed that Hb,RBC,S ,HCT,MCV,MCH,MCHC,PLT,S,PDW,MPV,PCTwere(14.2±1.85g/dl,4.89±0.55×10^6/ml,47.7±3.2%,83.5±8.48fl,29.1±2.78pg,37.5±2.64%,416.6±77.44×10^3µl,15.3±0.21%,10.4±1.35fl,0.24±0.05%)respectivelyinsmokers, while in non smokers group were (11.4±1.13g/dl, 4.87±0.39×10^6/ml,36.2± 3.04%, 72,7± 4.97fl, 25,2± 2.44pg, 31.6± 5.98%, 239×10^3± 71.02µl, 11.6± 1.2%, 8.9± 1.36fl, 0.24±0.06%) respectively . The result show that there is significant increased in Hb, HCT, MCV, MCH, MCHC, PLT,s ,PDW, and MPV in smokers P<0.05), while RBC,S and PCT didn't show significant difference between smokers and non smokers .

More ever the age had no effect on that parameters . The group of smokers also sorted into subgroups of (≥10 years , < 10 years) according to their back years of smoking , the effect of back-years smoking on blood values is also investigated and show no significant difference between two groups , also the group of smokers sorted into two subgroup according to the number of cigarette per day (≥ 10

cigarettes, < 10 cigarettes) , the effect of number of cigarette per day on blood values is also investigated and show no significant difference between two groups .

مستخلص الدراسة

تدخين السجائر تنطوي عليه مخاطر ذات تأثير كبير لمعظم الأمراض المزمنة، كما أن لديها آثار مزمنة وحادة على مكونات الدم. أجريت هذه الدراسة التحليلية الوصفية في ولاية الخرطوم من أبريل إلى ديسمبر 2014 لتحديد تأثير التدخين على بعض القيم الدموية على المدخنينالأصحاء. في هذه الدراسة مدخني السجائر من مائة مشارك من الذكور (50 مدخنين و50غير المدخنين)، وتتراوح أعمارهم بين سن 17-60 سنة ، وقد تم استخدام الاستبيان الهيكلي وأخذت منهم عينات من الدم.

كشفتنتائج هذه الدراسةأن الهيموقلوبين ,MCH, MCV, HCT كريات الدم الحمراء PCT,MPV,PDW,PLTS,MCHC, كانت 14.2±1.85g/dl, $4.89\pm0.55\times10^{6}$/ml,47.7±3.2%,83.5±8.48fl, 29.1±2.78pg, 37.5±2.64%,$416.6\pm77.44\times10^{3}$µl, 15.3±0.21%,10.4±1.35fl, 11.4±1.13g/dl , 0.24±0.05%)على التوالي في المدخنين ,بينما كانت في غير المدخنين $4.87\pm0.39\times10^{6}$/ml, 36.2± 3.04%,72,7± 4.97fl, 25,2± 2.44pg, 31.6± 5.98%,$239\times10^{3}\pm$ 71.02µl, 11.6± 1.2% ,8.9± 1.36fl , 0.24±0.06% على) ، MCH، MCV، HCTالتوالي . وتظهر النتائج أن هناك زيادة معنوية في الهيموغلوبين، PCT ، RBC,S) في حين$P<0.05$ لدى المدخنين (MPV، وPDW، ، PLT,S، MCHC لم تظهر فروق ذات دلالة بين المدخنين وغير المدخنين.أيضا تم تقسيم المدخنين إلي مجموعات فرعية (≤10 سنوات، <10 عاما) وفقا لسنوات التدخين، وقد تم أيضا دراسة تأثير سنوات التدخين على القيم الدموية وتبين عدم وجود اختلاف كبير بين هذه المجموعتين أيضا تم تقسيم مجموعة المدخنين إلى مجموعتين فرعيتين وفقا لعدد السجائر الذي يتم تدخينها في اليوم الواحد (≤ 10 سجائر، <10 سيجارة)، وتمت دراسة تأثيرها على القيم الدموية أيضا وأظهرت أيضا لا يوجد فرق كبير بين مجموعتين.

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Abbreviations

COPD	Chronic Obstructive Pulmonary Disease
FL	femtolitre
Hb	Hemoglobin
HCT	Hematocrit
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume.
MPV	Mean Platelet Volume.
PCT	Plateletcrit
PCV	Packed Cell Volume.
PDW	Platelet Distribution Width
Pg	Pictogram
RBC	Red Blood Cell
RNA	Ribosomal Nucleic acid.
WHO	Word Health Organization.

Chapter one

Introduction and literature review

Chapter one

Introduction and literature review

1. Introductions:

Hematology is the study of the normal and pathologic aspects of blood and blood elements .hematopoietic system is characterized by turnover and replenishment throughout life. The pluripotent hemopoitic stem cell (HSC) is the progenitor of the cells in blood . (Alvin andLilli ,2003).

The blood volume of an adult correlates with his or her (fat-free) body mass and amounts to ca. 4–4.5 L in women and 4.5–5 L in men of70 kg. The functions of blood include the transport of various molecules (O_2, CO_2, nutrients, metabolites, vitamins, electrolytes, etc.), heat(regulation of body temperature) and transmission of signals (hormones) as well as buffering and immune defense. The blood consists of a fluid (plasma) formed elements: Red blood cells (RBCs) transport O_2 and play an important role in pH regulation. White blood cells (WBCs) can be divided into neutrophilic, eosinophilic and basophilic granulocytes, monocytes, and lymphocytes. Neutrophils play a role in nonspecific immune defense, whereas monocytes and lymphocytes participate in specific immune responses. Platelets (thrombocytes) are needed for hemostasis. The formation of blood cells occurs in the red bone marrow of flat bone in adults and in the spleen and liver of the fetus. Hematopoietic tissues contain pluripotent stem cells which, with the aid of hematopoietic growth factors develop into myeloid, erythroid and lymphoid precursor cells. Since pluripotent stem cells are autoreproductive, their existence is ensured throughout life. (Despopoulosand Silbernagl (2003).

Tabacco smoking in various forms has been with us for more than 4 centuries . Inhalation of combusted tobacco via the means of smoking pipes, with or without water filtration ,cigars and cigarettes, with or without filters,has been the main route of tobacco consumption by humans .

Cigarette smoking has been associated with almost all common disease, including all forms of cancer, atherosclerosis ,myocardial infraction ,peripheral vascular disease ,pulmonary disease.(Owing (2005)

1.2 literature review

1.2.1 Blood components:

Blood is a very complex mixture of many types of component with very diverse properties and is often referred to as "liquid organ " . its distribution via the vascular system throughout the whole body is essential for the existence of the organism. (Schaller*et al*., 2008)

1.2.2 Main function of the blood :

1- Transport system of many type of component :
- Blood cells e.g. erythrocyte to transport oxygen
- Low molecular weight compounds e.g. salts and ions
- High molecular weight compounds e.g. proteins

2- Defense system against hostile pathogens such as bacteria , virus and fungi ,thus maintaining a balance between the organism and environment :
- Specialized cells e.g. lymphocyte monocyte and granulocyte
- Antibodies e.gIgG
- Component of the complement system

3- The wound sealing and wound healing system , life –saving precaution in the case of injuries :
- Blood cells, e,g blood platelets
- Blood coagulation and fibrinolysis

4- The balance of heat distribution throughout the body , thus guaranteeing a constant body temperature.

(Schaller*et al*., 2008)

1.2.3 blood constituents :

- Blood can be separated by sedimentation into two main parts :

1) The blood cells , which are primarily synthesized in the bone marrow , represent approximately 45% (by vol.) of entire blood .
2) The blood plasma or liquid portions of the blood represent approximately 55% of the entire blood (Schaller*et al*., 2008).

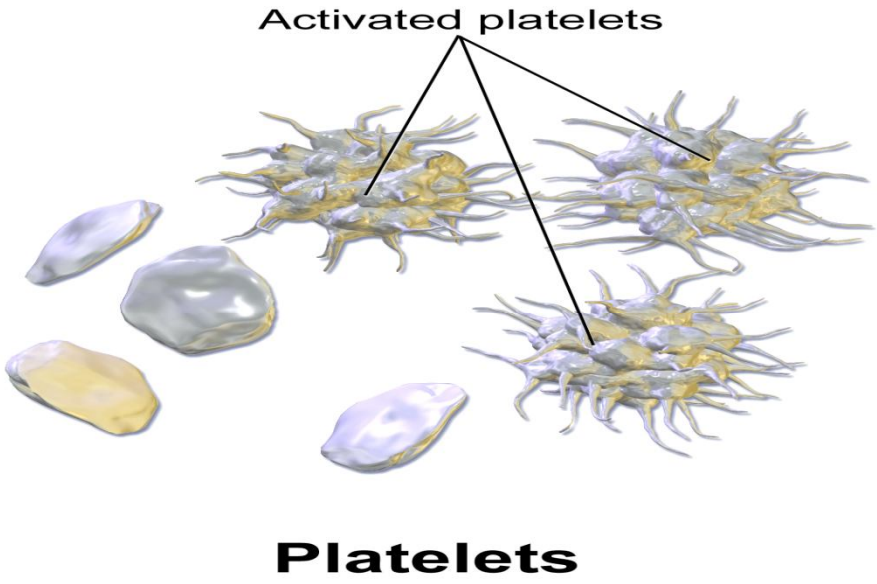
1.2.4 Platelets:

Megakaryocytes of the bone marrow are site of platelet formation. Diameter of a mature platelet is 2-3 µm, which usually remains alive for 5–9 days. Approximately 2/3 of the platelets circulate in the blood and 1/3 is stored in the spleen. The normal platelet count is (150–400) × 10^3 per microliter of blood. Each megakaryocyte can produce 5000–10000 platelets. An average healthy adult can produce 10^{11} platelets per day; old platelets are destroyed by phagocytosis in the spleen and liver (Kupffer cells). Platelets are unique in their structural assembly, though they are anucleate but have distinct mitochondria. Platelet plasma membrane, composed of phospholipid bilayer, is the site of expression of various surface receptors and lipid rafts which helps in signalling and intracellular trafficking. (Blair andLaumenhaft 2009).

1.2.4.1 Platelet Production:

Platelets are produced in the bone marrow, the same as the red cells and most of the white blood cells. Platelets are produced from very large bone marrow cells called megakaryocytes. As megakaryocytes develop into giant cells,

they undergo a process of fragmentation that results in the release of over 1,000 platelets per megakaryocyte. The dominant hormone controlling megakaryocyte development is thrombopoietin (often abbreviated as TPO). (James and George,2007)



{ Figure (1-1) Platelets} (James and George 2007)

1.2.6 Platelet Structure:

Platelets are actually not true cells but merely circulating fragments of cells. But even though platelets are merely cell fragments, they contain many structures that are critical to stop bleeding. They contain proteins on their surface that allow them to stick to breaks in the blood vessel wall and also to stick to each other. They contain granules that can secrete other proteins required for creating a firm plug to seal blood vessel breaks. Also platelets contain proteins similar to muscleproteins that allow them to change shape when they become sticky.

The pictures above show normal platelets on the left. They are shaped like a plate, therefore their name. When platelets are stimulated by a break in the blood vessel wall they change shape as shown in the other three pictures. They become round and extend long filaments. They may even look like an octopus, with long tentacles reaching out to make contact with the broken blood vessel wall or with other platelets. With these long filaments, platelets then form a plug to seal the broken blood vessel.(James and George 2007)

1.2.7Platelets function :

Primarily, platelet activity is associated with the initiation of coagulation cascades. Damage in blood vessel makes the sub endothelial surface the primary target site of platelet action, where it establishes the hemostasis. Various proaggregatory stimuli also known as platelet agonists promote the action of platelet adhesion to the subendothelial surfaces. During this process, platelet changes its shape, releases its granule contents, and gradually forms aggregates by adhering with each other .Thus its primary activity remains associated with minimizing blood loss.(Vinik*et al*.,2001)

1.2.8 Newer Platelet Parameters Generated by Automated Platelet Counters :

The electronic platelet counters can calculate three new platelet parameters. As of now these have a limited role in diagnosis(Bowles *et al*.,2005)

1.2.8.1Plateletcrit PCT:

-Plateletcrit is a measure of total platelet mass. Values vary depending on mean platelet volume resulting in overlap between normal platelets, thrombocytopenia

and thrombocytosis..Plateletcrit is an effective screening tool for detecting platelet quantitative abnormalities. Normal platelet count have a Plateletcrit within the range of 0.20-0.36%. (Chandrasekhar 2013)

1.2.8.2 Mean Platelet Volume (MPV) :

MPV is a reflection of megakaryocyte ploidy. MPV is increased in conditions associated with increased platelet turnover. The platelet mass remains constant in normal individuals.. The MPV falls with increasing platelet counts in a non-linear manner. The effect of storage in EDTA on MPV depends on the method used to perform the platelet count. The MPV increases if measured in an impedance counter because of a change in volume of platelets (see above). When measured by optical methods the MPV decreases nearly 10% possible because of a fall in the retractile index of the platelets because of dilution of cytoplasmic contents.(Bowles *et al*.,2005)

1.2.8.3 Platelet Distribution Width (PDW) : PDW is the variability in the size of platelets. Normally PDW increases with MPV. In patients with megaloblasticanaemia, aplastic anaemia and those on chemotherapy the relationship between MPV and PDW is lost. The patients show a low MPV but a high PDW. (Bowles *et al*.,2005)

1.2.8.4 Reference range of platelets indices :

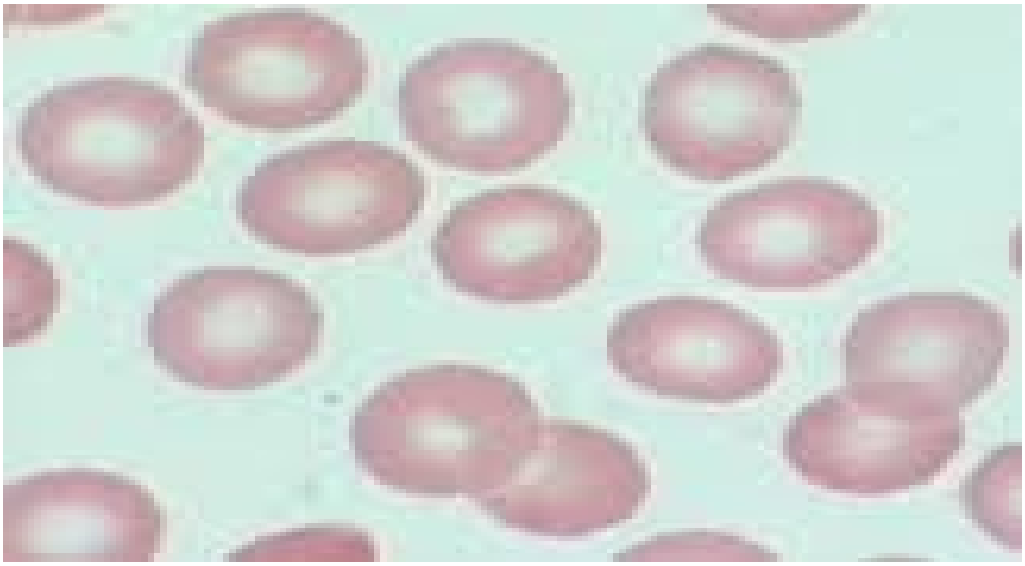
The average values obtained with expected ranges were (0.23-0.24%) for PCT, (12.39-13.19 fL) forMPV, and (46.61- 46.97%) forPDW(Wiwanitkit 2004).

1.2.9Erythrocytes :

The mature erythrocyte is unique in that it lacks a nucleus and organelles, and yet all component necessary for survival and function are present. The main cell component is hemoglobin. The erythrocyte is surrounded by a specialized membrane that allows it to transport O^2 and CO^2 and to survive in the circulation for approximately 120 days. (Martin *et al*., 1998).

1.2.9.1 Erythrocyte Life cycle:

Human erythrocytes are produced through a process named erythropoiesis, developing from committed stem cells to mature erythrocytes in about 7 days. When matured, in a healthy individual these cells live in blood circulation for about 100 to 120 days (and 80 to 90 days in a full term infant(**Harrison ,1979)**



(Figure (1-2) : Red Blood Cell ((Martin *et al*., 1998)

1.2.9. 2 Erythrocyte indices :

The erythrocyte count, Hb, and Hct can be utilized in calculations to determine the erythrocyte indices : mean corpuscular volume (MCV) ,mean corpuscular Hb (MCH) and mean corpuscular Hb concentration (MCHC). The indices are used both in quality control and in classifying and differentiating anemias . (Martin *et al*., 1998) .

1.2.9.2.1Mean corpuscular volume :

MCV indicate the average volume of a single erythrocyte in a given blood sample .it is expressed in SI units as femtoliters .

1.2.9.2.1. 1Calculation:

MCV ={Hct (%) x 10} / RBC (10^2 /L).

1.2.9.2.1.2 Terminology and interpretation :

Values of 80 to 100 fL are described as normocytic ,those less than 80 as microcytic ,and those more than 100 as macrocytic .

1.2.9.2.2Mean corpuscular Hemoglobin :

MCH indicates the mean weight of hemoglobin per erythrocyte ,expressed in SI units as pictograms .

1.2.9.2.2.1Calculation:

MCH = {Hb (g/dl) x 10} / RBC(10^{12} / L

1.2.9.2.2.2Terminology and interpretation :

Values of 26 to 34 pg are considered normal ,those less than 26 decreased , and those more than 34 increased .

MCH should correlate with with the MCV and MCHC. There is a higher MCH in macrocytic anemia because the erythrocytes are larger and carry more Hb. A lower MCH is found in hypochromic anemia and microcytic unless the erythrocytes are also spherocytic .(Martin*et al*., 1998).

1.2.9.2.3Mean corpuscular hemoglobin concentration :

MCHC indicates the average concentration of Hb in the erythrocyte in specimen. It is expressed in SI units as g/dl. Formalry MCHC was expressed in percent. (Martin *et al.,*1998)

1.2.9.2.3.1 Calculation:

MCHC ={Hb (g/dl) x100} / Hct (%)

1.2.9.2.3.2 Terminology and interpretation :

Values of 31 to 37 g/dl are considered normochromic , those less than 31 are hypochromic and those more than 37 are hyperchromic . (Martin *et al*., 1998) .

1.2.10Smoking :

Tobacco smoking is the inhalation of smoke from burned dried or cured leaves of the tobacco plant ,most often in the form of cigarette. People may smoke casually for pleasure ,habitually to smoking or in response to social pressure. According to

WHO about one –third of the world male population smokes tobacco (Haldane 1985).

1.2.10.1 Types of smoking

1.2.10.1.1 Cigarette

Cigarette smoking is the most common form of tobacco consumption.Because of curing process, smokers are mild enough to inhale in over close quantities, unlike cigar roll –your –own or pipe tobacco. Cigarette also contains number of additives, particular to enhance taste. (Haldane ,1985).

1.2.10.1.2 Roll-your-own:

Roll-your-own , often called rolley ups ,are very popular ,particularly in European countries ,these are prepared from loose tobacco ,cigarette papers and filers all bought separately –roll ups don't contain the addition (Haldane 1985)

1.2.10.1.3 Cigar:

A cigar is generally puffed , not inhaled-cigar common in many shapes and sizes .cigars generally come available in 2 categories in reference to color "natural" and "modure" . "natural" shades are ones that don't undergo further fermentation process , unlike "modure" which in its contraction involves further fermentation process to darken and strength then (in taste) the leaf (Haldane 1985).

1.2.10.1.4 Pipe

A pipe is for smoking typically consists of a small chamber (bowel) for combustion of the substance to be smoked and a thin stem (shank) that ends in moulpiece .pipe are made from a various other materials . (Haldane 1985)

1.2.10.1.5 Shisha or Hookah:

Shisha is a type of traditional Middle Eastern and South Asian water pipe,Which operates by water filtration and indirect heat ,it used to smoke hashish.

Typically tobacco is smoked from a hookah by placing richly flavored tobaccos in the smoking bowl, covering it with foil ,and placing a coal on top of foil. This keeps the tobacco from burning and allows it to bake. The resulting vapors are further cooled by the shisha water and filled by bubbling action in the basin of the hookah ,resulting in moist ,and worm light smoke (Haldane 1985).

1.2.10.2 The effect of smoking :

The effect of tobacco on health are significant depending on the way the tobacco is used (smoked snuffed or chewed) and the amount. Major health effect of smoking (the most common use of tobacco) includes an increase in lung cancer and cardiovascular disease(Narkiewicz*etal* ,2005).

Tobacco use leads most commonly to diseases affecting the heart, liver and lungs, with smoking being a major risk factor for heart attacks, strokes, chronic obstructive pulmonary disease (COPD) (including emphysema and chronic bronchitis), and cancer (particularly lung cancer, cancers of the larynx and mouth, and pancreatic cancer). It also causes peripheral vascular disease and hypertension. The effects depend on the number of years that a person smokes and on how much the person smokes. Starting smoking earlier in life and smoking cigarettes higher in tar increases the risk of these diseases. Also, environmental tobacco smoke, or secondhand smoke, has been shown to cause adverse healtheffects in people of all ages (Vainio , 1987).

1.2.10.3 The effect of smoking in hematological parameters :

Smoking has significant effect on many hematological parameters.Some effect transient and their severity varies between individuals as well as by the number and type of smoking ; smoking 10 cigarettes per day result in slightly higher Hb,PCV and MCV, this is probably consequence of the accumulation of carboxy hemoglobin in the blood together with decrease plasma volume. After single cigarette the carboxy hemoglobin level increases by about 1%. In heavy smokers the carboxy hemoglobin constituent 4-5% of the total Hb which may lead to polycythemia . Leucocytes count increase , largely as result of an increase in the neutrophil and neutrophili function also may affect , in addition to that lymphocyte count increase in some cases (increase in CD4 positive) (Lewis, *etal*2006).

Smoker tend to have higher platelets count more than non smokers, but the count decrease rapidly on cessation of smoking . Also nicotine increase the amount of cholesterol in blood which may cause the arteries to clog up with lafry tissue called thermal (Lewis, *etal*, 2006).

1.2.11Previous studies:

In TurkeyVarol*et al* (2013) studied the Effect of smoking cessation on mean platelet volume and reported that the platelet indices were assessed in regular smokers and control participants. platelet indices were measured at 3 months after smoking cessation in these 100 participants. The MPV values were significantly higher in smokers than those of controls (8.8 ± 0.9 vs 8.0±0.8 fL, respectively; P < .001). The MPV values decreased significantly at 3 months when

compared with the baseline values (8.9 ± 1.0 vs 7.9 ± 0.7 fL, respectively; P < .001). They have found that serum MPV values were significantly higher in regular smokers than in controls. serum MPV values decreased significantly at 3 months after smoking cessation.

In Turkey Besime*etal*(2014) studied the effect of smoking on healthy young men's hematologic parameters in 171 healthy male subjects between the ages of 20 to 30 years were investigated and reported that the MCV values of the group of smokers were higher than the values of non smokers, which were statically significant (p< 0.05) .

In India Anandha L S *etal* (2014) studied the effect of intensity of cigarette smoking on hematological and lipid parameters in 40 cigarette smokers and 40 non smokers and reported that there is significant increase in levels of hemoglobin ,hematocrite and red blood cells .

In india Jain k *et al*(2013) studied red blood cell parameters in smokers and nonsmokers with chronic periodontitis. A total of 77 males with chronic periodontitis were divided into 38 nonsmokers (group I) and 39 current smokers (group II). The RBC count, hemoglobin concentration, and packed cell volume were significantly lower in group II compared to group I. While the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were comparable in both the groups.

.

Rationale :

Cigarettes contain about 600 ingredients. When they burn, they generate more than 7,000 chemicals, according to the American Lung Association. Many of those chemicals are poisonous and at least 69 of them can cause cancer. Many of the same ingredients are found in cigars and in tobacco used in pipes and hookahs. According to the National Cancer Institute, cigars have a higher level of carcinogens, toxins, and tar than cigarettes.In the United States, the mortality rate for smokers is three times that of people who never smoked, according to the Centers for Disease Control and Prevention.

Objectives

General objectives :

Study of hematological parameter (Hb, RBCs count HCT,MCV,MCH,MCHC,Platelets count , PCT,MPV and PDW in Sudanese smokers in Khartoum state .

Specific objectives :

- To measure the effect of cigarette smoking on Hb , RBCs count , HCT,MCV,MCH,MCHC,PLATELETS , PCT,MPV and PDW.
- To compare HB,RBCs count, HCT,MCV,MCH,MCHC,Platelets count , PCT,MPV and PDW between cigarette smokers and non smoker.
- To determine the effect of pack years of smoking and number of cigarette taken per day on hematological parameter (Hb ,RBCs count ,HCT,MCV,MCH,MCHC,Platelets count, PCT,MPV and PDW).

Chapter two

Material and method

Chapter two

Materials and Methods

2.1 Study design:

This descriptive and analytical study carried out in Khartoum state during the period from (April –December 2014) to determine Hb,HCT, RBCs, MCV, MCH, MCHC, PLTs, PCT,MPV,PDW in cigarette smokers .

2.2. sample size :

Fifty samples were collected from Sudanese cigarette smokers and fifty sample collected from normal Sudanese as control group .

2.3 Sample area:

Khartoum state.

2.4. Inclusion criteria:

Healthy Sudanese smokers .

2.5 Exclusion criteria:

Presence of any other disease that may affect on hematological parameters .

2.6Tool of data collection:

The sample collected using structural l interviewing questionnaire.

2.7Sample collection:

2.7.1 Laboratory requirement:

- Automated hematological analyzer for determine RBC,S count ,HCT, MCV , MCH , MCHC , Platelets count , PCT ,MPV and PDW.
- EDTA Container
- Ethanol
- cotton
- Tourniquet
- syringe

2.7.2 Procedure of sample collection:

1-patient was either sat or lid down right on collection room.

2- The arm was positioned on the armrest so that the vein identified become under some tension and its mobility was reduced .

3- The skin was cleaned with 70% ethanol and allowed to dry .

4- Personal details were checked up on blood vials.

5- A tourniquet was applied to the arm .tight sufficiently to distend the vein , but not tightly to cause discomfort .

6- 3ml blood sample were taken from the superficial vein of the forearm.

7- Blood was collected in EDTA container, blood sample were analyzed by sysmex (Lewis *et al*., 2006)

2.8 Methods

2.8.1 Sysmex:

2.8.1.1Priciple of sysmex :

1- The reagent needed was checked for expiry date before use.

2- T he power switch was turned on ,self auto rinse background check was automatically performed and vend (vend for analysis) appeared number inputted by pressing sample number then number of sample was entered , the enter key was pressed .

3-sample was mixed sufficiently ,the tube was sited to the sample probe , and in that condition the start switch was pressed , when the LCD screen display analyzing ,the tube was removed.

4- After that the unit execute automatic analyses and the result was displayed in the LCD screen ,the result was printed out.

(Lewis *et al*., 2006)

2.9 Statistical analysis:

SPSS Software program for describe analysis of data ,(mean , standard deviations and P.value using T. test and Anova .

2.10 Ethical consideration:

Blood sample was collected after the participant were agreed after show informed consent.

Chapter three

Results

Results

One hundred healthy Sudanese were participate in this study. 50 smoker's represented case group and 50 non smokers represented control group. All participant fall in age ranged of 17-60 years old ; the most frequent group fall in range 15-35 (60.0%). According to gender all participants are male. The rate of cigarette among case group were most frequent about 1 to 10 cigarette per day (70%) and (30%) fall in range 11 to 20 .

The result of this study revealed that Hb ,RBC,S ,HCT,MCV,MCH,MCHC,PLT,S,PDW,MPV,PCTwere(14.2±1.85g/dl,4.89±0.55×10^6/ml ,47.7±3.2%,83.5±8.48fl,29.1±2.78pg,37.5±2.64%,416.6±77.44×10^3µl,15.3±0.21%,10.4±1.35fl ,0.24±0.05% respectively in smokers (table 3-1) while in non smokers group were (11.4±1.13g/dl, 4.87±0.39×10^6/ml,36.2± 3.04%, 72,7± 4.97fl, 25,2± 2.44pg, 31.6± 5.98%, 239×10^3± 71.02µl, 11.6± 1.2%, 8.9± 1.36fl, 0.24±0.06%) respectively table (3-1) . The result show that there is significant increased in Hb, HCT, MCV, MCH, MCHC, PLT,s ,PDW, and MPV in smokers were P.value (0.000) , while RBC,S and PCT didn't show significant defference between smokers and non smokers ,see table (3-1) . More ever the age had no effect on that parameters, see table (3-2). The group of smokers also sorted into subgroups of (≥10 years , < 10 years) according to their back years of smoking , the effect of back-years smoking on blood values is also investigated and show no significant difference between two groups,see table (3-3) , also the group of smokers sorted to two subgroup according to the number of cigarettes taken per day (< 10 cigarettes, ≥ 10 cigarettes) , the effect of number of cigarettes taken per day on blood values is also investigated and show no significant difference between two groups, see table (3-4) .

Table (3-1): The effect of smoking on hematological parameters among study population:

Variables	Smokers Mean ± SD	Non smokers Mean ± SD	p.value
RBCs($\times 10^6$/ml)	4.89 ± 0.55	4.87± 0.39	0.847
HB (g/dl)	14.2 ± 1.85	11.4 ± 1.31	0.000
HCT(%)	47.7 ± 3.2	36.2 ± 3.04	0.000
MCV(fl)	83.5 ± 8.48	72.7 ± 4.97	0.000
MCH(pg)	29.1 ± 2.78	25.2 ± 2.44	0.000
MCHC(%)	37.5 ± 2.64	31.6 ± 5.98	0.000
PLT($\times 10^3$ /µl)	416.6 ± 77.44	239.02 ± 71.02	0.000
PDW(%)	15.3 ±0.21	11.6 ± 1.2	0.000
MPV(fl)	10.4 ±1.35	8.99 ± 1.36	0.000
PCT(%)	0.25 ± 0.05	0.24 ±0.06	0.215

Table (3-2): Correlation between cigarette smoking for less or more than 10 years on some hematological parameters among smokers.

Variables	<10 years Mean ± SD	<10 years Mean ±SD	P.value
RBCs($\times 10^6$/ml)	4.87± 0.636	4.94 ±0.366	0.675
HB (g/dl)	14± 1.97	14.5 ± 1.59	0.400
HCT(%)	47.6± 3.29	47.8 ± 3.10	0.913
MCV(fl)	83 ± 9.73	85 ± 5.02	0.267
MCH(pg)	29± 2.98	29 ± 2.42	0.652
MCHC(%)	38± 2.51	37 ± 2.94	0.625
PLT($\times 10^3$ /µl)	418 ± 90.1	412 ± 45.6	0.767
PDW(%)	15.2± 0.20	15.3 ± 0.22	0.086
MPV(fl)	10.5± 1.3	10.3 ± 1.5	0.786
PCT(%)	0.26 ± 0.63	0.24 ± 0.42	0.459

Table (3-3): statistical correlation according to the Age group among smokers:

Blood parameters	15-35 year Mean± SD	36-55 year Mean± SD	P .value
RBCs($\times 10^6$ /ml)	4.94± 0.436	4.79 ±0.531	0.123
HB(g/dl)	13± 2.31	12.5 ± 1.83	0.343
HCT(%)	42± 6.36	41 ± 6.93	0.800
MCV(fl)	78± 8.68	78 ± 9.09	0.856
MCH(pg)	27± 2.91	28 ± 3.75	0.342
MCHC(%)	34± 5.83	35 ± 4.87	0.619
PLT($\times 10^3$ /µl)	327 ± 115.9	331 ± 117.3	0.867
PDW(%)	13.5± 2.17	13.4 ± 1.91	0.885
MPV(fl)	9.6± 1.65	9.9 ± 1.35	0.312
PCT(%)	0.25±0.67	0.24 ±0.58	0.342

Table (3-4): The effect of frequency of cigarette taken per day on hematological parameters among smokers .

Blood parameters	< 10 cigarettes Mean± SD	≥ 10 cigarettes Mean± SD	P.value
RBC,S($\times 10^6$ /ml)	4.8± 0.59	5.1 ± 0 .43	0.134
HB (g/dl)	13.9 ±1.69	15.0±2.00	0.042
HCT(%)	47±3.27	48±3.09	0.578
MCV(fl)	82±9.45	85±5.23	0.222
MCH(pg)	29±2.92	29±2.49	0.522
MCHC (%)	37±2.32	38±3.27	0.261
PLT,S ($\times 10^3$ /µl)	416±85.7	416±55.9	0.982
PDW (%)	15.3± 0 .19	15.3±.25	0.711
MPV(fl)	10.5±1.34	10.3±1.4	0.682
PCT (%)	0.26 ± 0.06	0.26± 0 .02898	0.618

Chapter four

Discussion , conclusion and recommendation

Chapter four

Discussion, conclusion and recommendation

4.1Discussion

This is a descriptive case control study, conducted to measure the RBCs, hemoglobin, HCT, MCV, MCH, MCHC, platelets counts, PDW, MPV and PCT among smokers and non smokers.

This study demonstrated that, RBCs, hemoglobin, HCT, MCV, MCH, MCHC, platelets, PDW, MPV and PCT were significantly higher in the smokers than in control group. This result was in agreement with Varol*etal*(2013)who reported that MPV was significantly higher among smokers; also Jain K et al(2013); Their studies demonstrated that, hemoglobin concentration, and packed cell volume were significantly increased among smokers and disagreed with study conducted by Mercelina-Roumans*et al* ((1995)who showed that there was no significant difference between the platelet count in the two groups.

According to our study there was insignificant association between RBCs and smokers this result was not similar to study carried out by Jain et al(2013); who also reported that there was significant association between RBCs and smokers. The differences between our result and published studies may due to different in sample size and method used in determination.

Our study agreed with Mercelina-Roumans*et al*(1995),in which their study demonstrated that the hematological parametersamong smokers were insignificantly correlate with age groups.

As a result of subgroup analysis according to the duration of of smoking per years our result agreed with besime I (2014) which show that there was no significant difference on (Hb, HCT,MCV,MPVand platelets between the two subgroup .

Also our study agreed with Anandha L *et al*(2014) in which there is significant increase in levels of hemoglobin and hematocrit.

The excessive carbone monoxide (CO) exposure may produce polycythemia in humans as well in animals .The half life of the CO in the body is 3-5 hrs . In a person who smokes frequently and continuously ,thecarboxyHb levels increases and produces a progressive hypoxemia and as the CO binds with Hb functional anemia is produced .

This causes the impaired oxygenation of tissues and change in hematological parameters .

4.2 Conclusion:

Increase of hemoglobin , hematocrit, MCV, MCH, MCHC, PLT,s ,PDW, and MPV were found significant among smokers than in nonsmokers , which were statistically significance ($p< 0.01$)while RBC,s and PCT show insignificant differences .As a result of subgroup (cigarette smoking for less or more than 10 years) the hemoglobin ,RBC,s hematocrit , MCV,MCH,MCHC.MPV,PDW were insignificantly increased ,also that hematological parameters wereinsignificantly increased according to the number of cigarette taken per day (< 10 cigarettes, ≥ 10 cigarettes). More ever the age had no effect on that parameters.

4.3 Recommendations:

- Smokers should be informed about the danger and the effect of smoking on hematological parameters and on health at all and advice them to stop smoking .
- smokers should be periodically checking the hematological parameters.
- Further studies should be done to assess all hematological parameters among different types of smoking.

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Appendix

Appendix

Appendix (1): Questionnaire

Sudan University of Science and Technology

Hematology department

Questioner about

Smoker Cigarette

Sample NO( ):-

-Name ----------

-Age ----------

-sex----------

-Non smoker cigarette----------

-Smoker cigarette----------

The period of smoking ----------

The number of smoke per day----------

Lab investigation:-

Hb:

RBCs:

PCV:

MCV:

MCH:

MCHC:

PCT:

MPV:

PDW:

Signature:-Date:-

Appendix (2):

Cigar

Appendix (3) :

Sysmex

Appendix (4):

Dangers of smoking