Sudan University of Science and Technology

College of Graduate Studies

كلية الدراسات العليا

Assessment of Platelet Count and Platelet Indices among Sudanese Smokers in Khartoum state.

تقييم عدد الصفائح الدمويه ومؤشراتها لدى المدخنين السودانيين في ولايه الخرطوم

A dissertation Submitted in Partial Fulfillment of the Requirement for the M.Sc. Degree in Medical laboratory Science (Haematology and Immunohaematology)

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الآيه

قال تعالى:

بسم الله الرحمن الرحيم

﴿إِنَّ الْمُتَّقِينَ فِي جَنَّاتٍ وَنَهَرٍ (54) فِي مَقْعَدِ صِدْقٍ عِندَ مَلِيكٍ مُّقْتَدِرٍ (55)﴾

صدق الله العظيم

سورة القمر

Dedication

I dedicate this research to

My father

My mother

My husband

My brothers and sister

My friends and colleagues

And

Everyone who facilitated this work

Acknowledgement

First of all thanks for **Allah** Almighty for helping me to complete this work.

And thanks to my supervisor

Dr. **Munsoor Mohammed Munsoor**

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Al-Rebat Medical Specialize Complex staff

Iam greatfull to my friends **Faiza Salah**, **Esraa Abo obeida** , **Ayat Mohamed** who helped me during the preparation of this work .

And for all the **smokers and controls** from whom the blood sample were collected.

Abstract

This is analytical case control study conducted in Khartoum state on smokers Sudanese people as study group and healthy volunteers as control group in Al-Rebat Medical Specialist from september to november 2018.

Forty healthy smoker's male as (cases) and forty healthy non-smokers male as (controls) were informed about study agreed for participation. Two and half ml of EDTA venous blood was collected and analyzed automatically (URIT- 3010) to measure platelets count and platelets indices (MPV, PDW, PCT, P-LCR) The data analyzed using SPSS(version 16)computerized program.

All participant age was between (18-45) years. (18—25) was the most frequent age group within smokers and non-smoker (65%, 71%) respectively. while the age group (26—45) was the least frequent in both study subject (30 %, 29%).

Within smokers (85%) was smoking less than 10 year while (15%) was smoking more than 10 years (15%)and (50%) of smokers get 10 and less cigarette per day and (50%) of smokers get more than 10 cigarette per day

Results showed that the platelet count and platelet indices (MPV , PDW , PCT , P-LCR) were insignificant different between cigarette smoker and non-smoker (P-value 0.560 , 0.285 , 0.638 , 0.325 and 0.470 respectively).

المستخلص

هذه دراسة تحليليه حاله وحاله ضبط أجريت على الأشخاص السودانيين المدخنين في ولاية الخرطوم كمجموعة دراسية وأصحاء غير مدخنين كمجموعة ضبط وقد اجريت هذه الدراسة في مجمع الرباط الطبي التخصصي اجريت الدراسه في الفتره من سبتمبر إلى نوفمبر 2018 لتقييم تاثير التدخين علي الصفائح الدمويه ومؤشراتها.

تم ابلاغ المشاركين بهدف الدراسه واخذت الموافقه منهم شفاهة , أربعون من المشاركين الذكور الاصحاء كانو مدخنين (حالات) وأربعون من المشاركين الأصحاء من الذكور كانو غير مدخنين (الضوابط). تم جمع اثنان ونصف مل من الدم الوريدي في مانع التجلط EDTA ومن ثم تم اختبارها بواسطه جهاز عد الدم الالي لقياس عدد الصفائح الدمويه ومؤشرات الصفائح الدمويه لدي المشاركين و ومن ثم تم تحليل النتائج باستخدام برنامج الحزم الاحصائيه للعلوم الاجتماعيه (الاصدار 16).

تتراوح اعمار المشاركين من (45-18) سنة. (25-18) سنة كانت الفئة العمرية الأكثر شيوعًا بين المدخنين وغير المدخنين (65٪ ، 71٪) على التوالي. في حين كانت الفئة العمرية (45-26) سنة هي الأقل تكررا في كلا المجموعتين (30% , 29%).

85%)من الحالات المشاركه كانت تدخن لمده اقل من عشر سنوات و(15%) كانوا مدخنين لاكثر من عشر سنوات كما ان (50%) كانوا يدخنون اقل من 10 سيجاره باليوم و (50%) كانوا يدخنون اكثر من 10 سيجاره باليوم

تظهر النتيجة أن عدد الصفائح الدموية ومؤشرات الصفيحات لم يكن فيها فرق ذو دلاله معنويه بين المدخنين وغير المدخنين

(Plt count, MPV، PDW، PCT (P-value 0.560 ،P-LCR) 0.285، 0.638، 0.470 , 0.325) على التوالي .

List of content

List of Tables

Abbreviations

ADP   Adenosine diphosphate

APTT   Activated parteial thromboplastin time

ATP   Adenosine triphosphate

CBC   Complete blood count

DNA   Deoxy ribonueclic acid

EDTA   Ethyl diamine tetra acetic acid

Fl   Fimtoleter

Hb   Heamoglobin

Hct   Heamatocrit

HMWK   Hight moleculer weight kininogen

GP   Glycophorin

MCV   Main corpuscular volume

MPV   Main platelet volume

PCT   Plateletcrit

PCV   Packed cell volume

PDW   Platelet distribution width

Plt   Platelets

P-LCR   Platelet large cell ratio

PT   Prothrombin time

RBC   Red blood cell

SPSS   Statistical package of social science

VWF   von Willebrand factor

WBC   White blood cell

WHO   World health organization

Chapter one

Introduction and Literature review

Chapter one

Introduction and Literature review

1.1 Introduction:

Platelets (thrombocytes) are a nucleated small discoid blood cells and considered a very high energy cell with metabolic rate 10 times that of an erythrocyte. It is originated from cytoplasmic fragments of bone marrow megakaryocyte, with a diameter of 1.5 – 3.5 μm and volume up to 10.4 fl (Turgeon, 2017).

Each megakaryocyte giving rise approximately to 1000 – 5000 platelets into the circulation where they have 9.0 ± 1 day survival time. Platelet has an active role in the vascular integrity repair and primary hemostasis (Bashir,Dirar, et al 2017) .

Platelet indices including mean platelet volume (MPV), platelet distributing width (PDW), platelet large cell ratio (P-LCR) and plateletcrit (PCT) offer valuable information about the morphology and maturity of platelets. MPV is a measurement of the average size of platelets in blood (Lewis, 2006).The higher MPV is a larger platelets size. PDW reflects the variability in the platelets size and it's therefore increased in the presence of platelets anisocytosis. PCT is an expression of a percentage that reflects the volume occupied by platelets in blood. PCT is directly related to the platelet count and the size of the platelets (Wiwanitkit, 2004). It has been suggested that platelet indices are potentially useful markers for the early diagnosis of many types of diseases, can play a role in the rapid evaluation of bone marrow activity of patients with platelet-associated disorders MPV reflects an atherosclerothrombic tendency in the human body MPV and P-LCR are indicators of active bone marrow. On the other hand, PCT and PDW can be used to differentiate reactive thrombocytosis from myeloproliferative disorders (Abass, Ismail, et al, 2016).

Smoking is important health problem cigarette contain about 6000 chemical substance, it exerts pharmaco- logical, mutagenic, cancerogenic, toxic, and inflammatory effects (Al-Azzawy, 2011). Cigarette contains carcinogens (polycyclic aromatic hydrocarbons etc.), irritant substances, nicotine, carbon monoxide, and other gases. Cigarette smoke contains many oxidants, and free radicals which can harm lipids, proteins, DNA, carbohydrates, and other biomolecules. The effects of smoking on various metabolic, and biological processes, hormone secretion, and hematopoietic system have been demonstrated (Inal, Hacıbekiroglu, et al 2014).

Smoking has been also identified as a principal underlying etiology for the occurrence and progression of cardiovascular diseases, inflammatory disorders, and oxidative stress stimulation. Cigarette smoking crucial role in disrupting platelet activation and aggregation, as well as other coagulation processing components leading to thrombotic formations has been recently suggested (Ghahremanfard, 2015).

Previous studies have demonstrated that chronic smoking causes endothelial dysfunction, platelet activation and hemostatic disorder (Varol et al, 2013).

1. 2 Literature review

Haemostasis is an efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury and prevent extensive clots developing (Hofbrand, 2006).

The haemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms

There is five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels (Hofbrand, 2006).

1.2.1 Platelets:

Platelets are produced from bone marrow precursors known as megakaryocyte Normal platelet count ranges from 1, 50,000 – 4, 00,000/cu mm (150–400 × 109/L) and it has a normal life span of 8-10 days (Turgeon ,2017).

1.2.1.1 Morphology of platelets

by Light microscopy on Romanowsky stained peripheral blood smear platelets appear as small (diameter of 2-3 μm, approximately 1/5 the diameter of red blood cell), round, anuclear cells with prominent reddish purple granules (Ramadas 2012).

by Electron microscopy platelets reveal several glycoprotein receptors, two types of cytoplasmic granules and a contractile cytoskeleton Glycoprotein receptors.

The two main platelet membrane receptors are Glycoprotein (Gp) IIb-IIIa: It is the main receptor on cell surface.

Glycoprotein Ib-IX: It is a receptor for binding vWF with platelet (Ramadas, 2012).

Cytoplasmic granules: Alpha (αα αα α) granules contain fibrinogen, factors V and VIII, von Willebrand factor (vWF), fibronectin, chemokines, platelet

factor 4 (heparin-binding chemokine), platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β). They also have adhesion molecule P-selectin on their membranes. Dense granules contain potent aggregating molecules adenosine diphosphate (ADP), ATP, serotonin, ionized calcium, histamine and epinephrine (Ramadas, 2012).

Contractile cytoskeleton: It consists of dense microtubules and circumferential microfilaments, which maintain the disk shape of platelets (Ramadas, 2012).

Role of platelets in hemostasis form the primary hemostatic plug and release platelet activating and procoagulant molecules. And provide a procoagulant surface for the activation of coagulation system (Hofbrand, 2006).

1.2.2 Blood vessel wall:

Endothelial cells in the blood vessel wall, particularly endothelial cells play an important role in the regulation of homeostasis. The normal intact smooth endothelial cells lining the blood vessels inhibit platelet activation, prevent coagulation and enhance fibrinolysis. The endothelial cell, thus presents as a nonthrombogenic surface and maintains blood in a fluid state. Endothelial cells use three antithrombotic systems to limit coagulation. These are antithrombin III, protein C and protein S, tissue factor pathway inhibitor (TFPI). The injured endothelium or under inflammatory conditions, it down regulates its anticoagulant functions and becomes procoagulant. Injury to endothelial cells or endothelial activation exposes highly thrombogenic subendothelial extracellular matrix (ECM), which leads to platelet adherence and activation. There is simultaneous activation of coagulation system (Ramadas, 2012).

Vasoconstriction following injury, blood vessels also undergo vasoconstriction which assists in arresting the blood loss. Vasoconstrictors may also be produced by endothelial cells (angiotensin II) and activated platelets (thromboxane A2)

 (Turgeon, 2017).

1.2.3 Coagulation system:

Coagulation factors or clotting factors are transported in the plasma as procoagulants. During coagulation, the procoagulants become activated to produce thrombin. Thrombin converts the soluble fibrinogen into insoluble meshwork of fibrin gel.

Traditional coagulation pathways coagulation can be activated by two pathways namely extrinsic and intrinsic. Both the pathways converge on the activation of factor X (Ramadas, 2012).

1.2.3.1 Extrinsic Pathway:

It requires an exogenous trigger; hence the name extrinsic, but now it is observed that released tissue factor from vascular injury can initiate this pathway, tissue factor is present on the vascular wall and is not normally in contact with blood, whenever there is vascular injury, the tissue factor (also known as thromboplastin or factor III) is liberated at the site and triggers coagulation by this pathway, the coagulation factor utilized in extrinsic pathway is factor VII. The prothrombin time (PT) is the laboratory test which assesses the function of the coagulation factors involved in the extrinsic pathway (factor VII) and common pathway (factors X, II, V and fibrinogen). PT is performed by adding tissue factor (Mackman ,teilly ,et al, 2007).

1.2.3.2 Intrinsic Pathway:

It is activated by exposure of factor XII (Hageman factor) to any thrombogenic surfaces (including glass beads), the coagulation factors utilized in intrinsic pathway in order of reaction are: factors XII, prekallikrein HMWK, XI, IX and VIII.,The partial thromboplastin time (PTT)/activated partial thromboplastin time (APTT) assesses the function of the coagulation factors utilized in the intrinsic pathway (factors XII, pre-K, HMWK, XI, IX and VIII) and common

pathway (factors X, V, II and fibrinogen). PTT is initiated by adding negatively charged particles like glass beads (Ramadas, 2012).

1.2.3.3 Common Pathway: Above two pathways have in common, factors X, V, prothrombin and fibrinogen; and this part of coagulation pathway is known as the common pathway. This division is arbitrary because there are many interconnections between these two pathways (Ramadas, 2012).

1.2.4 Coagulation regulatory mechanism:

The activated coagulation system must be limited to the site of vascular injury so as to prevent coagulation in the entire vascular system.This is achieved by three endogenous anticoagulants:

Antithrombin: It inhibits the activity of thrombin and factors IXa, Xa, XIa and XIIa. One of the antithrombin is antithrombin III which gets activated by binding to heparin-like molecules on endothelial cells. The heparin used to minimize thrombosis acts by activating antithrombin III (Ramadas, 2012).

Proteins C and S these are vitamin K dependent proteins that act as a complex. Thrombin generated by activation of coagulation cascade, binds with thrombomodulin present on the endothelial cell membrane and activates the coagulation regulatory system called protein C system. Activated protein C (APC) binds with protein S to form APC-protein S complex. This complex inactivates factors Va and VIIIa (Esmon, 2000).

Tissue factor pathway inhibitor (TFPI): TFPI is a protein which inactivates tissue factor– factor VIIa complexes and inactivates factor VIIa (Mackman, teilly ,et al ,2007).

1.2.5 Fibrinolytic system:

Activation of the coagulation also initiates fibrinolytic system, so that the size of the clot is limited. Fibrinolytic system does this by removal of fibrin from the

clot. Otherwise the clot may progress and involve the entire circulation with its consequences. In the fibrinolytic system, plasmin is formed from the inactive circulating precursor plasminogen. Plasminogen may be activated either by: factor XII–dependent pathway or plasminogen activators (PAs): The most important of the PAs is tissue plasminogen activator (t-PA) secreted by endothelial cells and others include urokinase, free plasmin is rapidly inactivated by binding to a plasma protein named α2-antiplasmin, thereby limiting the action of plasmin. Plasmin cleaves fibrinogen and fibrin and produces number of fibrin degradation (FDP) products, also known as fibrin split products (fragments like X, Y, D, E and D-D). These FDPs clear minor clots in the vessels and restore the blood flow. Elevated levels of FDPs, most notably D-D fragment, known as D-dimers is used as a marker for thrombosis and is used for the diagnosis of disseminated intravascular coagulation (DIC). The activity of t-PA and urokinase is controlled by releasing plasminogen activator inhibitor-1 (PAI) secreted by endothelial cells (Ramadas, 2012).

1.2.6 Primary hemostasis:

Hemostasis is the body's response to vascular damage and includes several sequences of events at a site of vascular injury normally, blood vessels are lined by a smooth, nonthrombogenic endothelium. Injury to the endothelium causes transient vasoconstriction and exposes highly thrombogenic subendothelial extracellular matrix (ECM), initiating the platelet events. These events forms hemostatic plug and prevents any further hemorrhage at the site of injury (Ramadas, 2012).

Primary hemostatic plug different events in platelet activation are:

1.2.6.1 Platelet adhesion and shape change:

Initial step is adhesion of platelets to subendothelial structures at the site of injury. The link is mainly through receptor sites (GpIb-IX) on the platelet with

subendothelial von Willebrand factor (vWF). vWF is synthesized by both endothelial cells and mega- karyocytes.

Platelets change their shape from round to spherical to stellate, thereby markedly increasing the surface area. (Hofbrand, 2006)

1.2.6.2 Platelet secretion (release reaction):

Soon after adhesion, platelets release granule contents which contain pro-aggregatory substances like ADP, serotonin (5-hydroxytryptamine), fibrinogen and vWF. Calcium is also released and is required for the coagulation (Hofbrand, 2006).

1.2.6.3 Platelet aggregation:

The secreted products recruit additional platelets and cause aggregation to each other through the receptor sites (Gp IIb-IIIa) using fibrinogen as an intercellular bridge, these clumps of platelets so formed quickly stop bleeding from the site of injury and are known as primary hemostatic plug (Hofbrand, 2006).

1.2.7 Platelet indices:

The platelet indices, plateletcrit, mean platelet volume, platelet distribution width, platelet large cell ratio, and platelet large cell concentration are a group of platelet parameters related to platelet morphology and proliferation kinetics.

(Budak,Potal, 2016).

1.2.7.1 MPV (mean platelet volume):

It is the calculated measurement of the average size of platelet found in blood. The normal range is given as 7.5 – 10.4 fl (Larsen, et al, 2014).

A high MPV is usually a sign that there are more young platelets circulating in the bloodstream. As in response to surgery, bone marrow releases more of the young,larger platelets, and MPV rises, alow MPV along with a low platelet

count can point towards disorders affecting the bone marrow that slow down or decrease the production of platelets, such as a condition called aplastic anemia.In addition, a low MPV can be seen with high, low or normal platelet counts in sepsis (a life-threatening reaction to infection in the body), splenomegaly (enlarged spleen), chronic kidney failure, or treatment with drugs that suppress blood production (Chandrashekar, 2013).

1.2.7.2 PDW (platelet distribution width):

It is an indicator of volume variability in platelet size, changes with platelet activation and reflects the heterogeneity in the thrombocyte morphology (anisocytosis). Normal rage given is 9 – 13 fl (Sachdev ,Tiwari, et al, 2014).

Platelets recently released from bone marrowtend to be larger and to contain more RNA than older, smaller platelets, which discard their endoplasmic reticulum as they mature, the volume is determined by a machine and a complete blood profile, known as a CBC, this reading determines if a patient's body is producing larger than average platelets, indicative of platelet destruction or bone marrow diseases.

 (Chandrashekar, 2013).

1.2.7.3 PCT (Plateletcrit):

It is the volume (total platelet mass) occupied by platelets in the blood stream as a percentage volume of PCT varies depending on MPV and platelet count according to the formula PCT= PLT x MPV/10.000 (Charadrashekar, 2013).

 Values vary depending on mean platelet volume resulting in overlap between normal platelets, thrombocytopenia and thrombocytosis .with normal range (0.10_0.28%) (Charadrashekar, 2013).

1.2.7.4 P-LCR (Platelet large cell ratio):

It is an indicator of circulated large platelets in size (> 12 fl), sometimes used to monitor the platelet activity. It is presented in percentage of normal range 15 – 35% (Wiwanitkit, 2004).

Increased percentage of large platelets (P-LCR) is observed in patients with Hyperlipidaemia and suggest possible risk of thrombosis.An increase in P-LCR + MPV + PDW has been observed in autoimmune thrombocytopenic purpura (Bashir, Dirar,et al, 2017).

Platelet indices definition PI are group of platelet parameters determined together with automated complete blood count (CBC) profiles .

Parameter	Discription	Unit
Platelet	Measure of the thrombocyte count	Cell/ Microliter (µl)
MPV	Measure the thrombocytes volume	Femtoliter (fl)
PDW	Indicator of variability in size and activation	Femtoliter (fl)
PCT	The volume occupied by platelets in the blood	Percentage (%)
P-LCR	Indicator of large platelet in the blood	Percentage (%)

1.2.8 Smoking

1.2.8.1 Definition of smoking:

Smoking is the inhalation of the smoke of burning tobacco encased in cigarettes, pipes, and cigars (Medical dictionary, 2007).

A smoking habit is a physical addiction to tobacco products. Many health experts now regard habitual smoking as a psychological addiction, too, and one with serious health consequences (Medical dictionary, 2007).

1.2.8.2 Component of cigarettes

Cigarettes, tobacco are made from dried tobacco leaves. Other substances are added for flavor and to make smoking more pleasant. The smoke from these products is a complex mixture of chemicals produced by burning tobacco and its additives (Baker, Ainsworth, et al, 2000).

Tobacco smoke is made up of thousands of chemicals, including at least 70 known to cause cancer. These cancer-causing chemicals are referred to as carcinogens. Some of the chemicals found in tobacco smoke include:

Nicotine (the addictive drug that produces the effect people are looking for and one of the harshest chemicals in tobacco smoke)

Hydrogen cyanide, formaldehyde,lead, arsenic, ammonia, radioactive elements, such as uranium , benzene, carbon monoxide, nitrosamines and polycyclic aromatic hydrocarbons (PAHs) (US Food and Drug, 2012).

1.2.8.3 Effect of smoking:

Acigarette smoker is exposed to a number of harmful substances including nicotine, free radicals, carbon monoxide and other gaseous products. It is widely known that smokers have higher risk for cardiovascular diseases, hypertension, inflammation, stroke, clotting disorder, and respiratory disease (Gitte, 2011). Moreover, cigarette smoking accelerates pathogenesis in different type of cancers such as lung, pancreas, breast, liver and kidney. Similarly, it also enhances pH in stomach that resulted in peptic ulcers and gastric diseases. (Asif, karem, et al,2013).

The major health effects of cigarette smoke include: cancer; noncancerous lung diseases; atherosclerotic diseases of the heart and blood vessels; and toxicity to the human reproductive system. Retardation of healing of peptic ulcers and interaction with certain therapeutic drugs (Harris, 1996).

1.2.8.4 Effect of smoking on hematological parameter:

Smoking has significant effect on hematological parameter and these depend on duration and severity of smoking, smoking about 10 cigarete per day result in slightly increased in Hb, PCV and MCV this is probaply of the accumulation of carboy hemogloinin the blood together with decreased plasma volume

(lewis ,dacie,2006).

Study show increase in White blood cell, neutrophil, basophil and eosinophil counts in smoker. Mean corpuscular volume, red cell distribution width and neutrophil/lymphocyte ratio were also higher in smokers when compared with nonsmoker (Tulgar, kakarn, et al ,2016).

 Comparing platelet indices across smokers and non-smokers showed that the mean platelet count was statistically significantly higher in adult smokers (Ghahremanfard, Semmai, et al 2015).

 The MCV values of the smokers were increased and the white blood cell (WBC) counts of the individuals smoking for 5 or more years were significantly higher than those with a history of smoking less than5 year (Inal, Hacıbekiroglu, et al 2014).

1.3 Rationale

Smoking is the most important public health problem. Many studies performed have proved its deleterious effects on many organ systems mainly respiratory, and cardiovascular systems

According to the World Health Organization, smoking leads to approximately 6 million preventable deaths worldwide, each year. As of 2030, it is estimated that this number will be more than 8 million (Who,2011). Apart from causing cancer, smoking can lead to cardiovascular, neurological and respiratory diseases and may affect every system. Although the detrimental effects of smoking are associated with the number of years and amount of use, the effect of smoking may be reversible and mortality decreases, even if smoking is ceased at a later age

(Tulgari,Cakar, et al,2016).

In Sudan Smoking prevalence, males (% of adults) was reported at 23.84 % in 2009, according to the World Bank collection of development indicators.

Smoking has been also identified as a principal underlying etiology for the occurrence and progression of cardiovascular diseases, inflammatory disorders, and oxidative stress stimulation (Padmavathi,Redy, et al 2010).

Cigarette smoking's has crucial role in disrupting platelet activation and aggregation, as well as other coagulation processing components leading to thrombotic formations has been recently suggested (Ghahremanfard, Semmai, et al 2015).

Because the blood platelet appears to play the central role in the initiation of thrombosis, this study conducted to define the effect of smoking on platelet count and platelet indices is seems important.

1.4 objectives

1.4.1 General objective:

To evaluate the effect of the cigarette smoking on platelet count and platelet indices in Sudanese men in Khartoum state.

1.4.2 Specific objective:

1/ to estmate the effect of the cigarette smoking on platelet count and platelet indices in Sudanese men .

2/ to compare the mean of PLT Count and PLT indices (PCT, MPV, PDW, P-LCR) between the case study and control study.

3/to assess the effect of number of cigarette/day and duration of smoking on PLT count and PLT indices if there was different in parameter betwaan cases and control group.

Chapter Two

Materials and methods

Chapter Two

Materials and methods

2.1 Study Design:

This a case control study conducted during (September 2018 to November 2018).

2.2 Study Area:

Khartoum state

2.3 Study Population:

Forty samples from Sudanese healthy smoker and forty samples was collected from healthy nonsmoker as control.

2.3.1 Inclusion criteria:

-Healthy and smoker male as case group.

-Healthy nonsmoker individuals as control group for comparing.

2.3.2 Exclusion criteria:

Any smoker have diabetic mellitus, hypertension, and blood transfusion, take aspirin or alcohol user.

2.3.3 Data Collection:

The data Collected using nonself administrated questionnaire which was specifically designed to obtain information that helped in study

2.3.4 Sample collection:

Venous blood was collected using sterile disposable plastic syringes after cleaning the vein puncture area with 70% ethanol , blood was add to the anticoagulant at ratio of 2.5 to 1.5 of 0.1%EDTA solution and gently mixed .

2.4 Methodology:

2.4.1 CBC (Automated sysmix technique):

2.4.1.1 Principle of sysmix:

The principle is based on the following:

Particles suspended in an isotonic diluents, when drawn through an aperture which has an electric current flowing through it will cause a measurable drop in voltage which is proportional to the size of the particle passing through the aperture is constant the particle can be quantified per unit volume. This is also called electrical impedance (Mark, 2016).

2.4.1.2 Methods of sysmix:

Whole blood mode:

Blood is aspirated from the sample probe into the sample rotor valve:

1. 4.0 µl of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 µl of diluents and brought to the mixing chamber as diluted sample (1st step dilution).

2. Out of the 1:500 dilution sample 40 µl is measured by the sample rotor valve, diluted into 1:25000 with 1.960 µl of diluent then transferred to the RBCs/plt transducer chamber (2nd step dilution).

250 µl of the sample in the RBCs/Plt transducer chamber is aspirated through the aperture. At this time RBCs and Plt are counted by the DC

detection method. At the same time, hematocrit (Hct) value is calculated by RBCs pulse height detection method (Mark, 2016).

2.5 Ethical consideration:

It was considered that all information obtained from participants was kept as highly confidential data and specimen's results were not permitted.

The participators were provided with information about the study and any risk which may be raised especially when the collection technique was applied.

2.6 Data analysis:

SPSS (statistical package of social science) version 16 software programs was used values were given as mean ± SD. platelet count and parameters were evaluated using independent T test (to compare the mean of parameters between the study group and control group and to compare the mean of parameters result between the study group in different duration groups and different number of cigarette per day groups.

Chapter Three

Results

Chapter Three

3.1 Results

40 smokers (50%) and 40 non-smoker (50%) have participated in this study all were male .(table 3.1).

Within smoker group there were 20 (50%) smoker get 10 and less cigarette per day and 20 (50%) smoker get more than 10 cigarette per day. (Table 3.2)

34 smoker were smoking less than 10 year (85%) while 6 smoker was smoking more than 10 years (15%). (Table 3.3)

There was no statistical different between smokers group compare with control group in platelet count , MPV , PDW , PCT and P-LCR .(P-value 0.560 , 0.285 , 0.638 , 0.325 , 0.470 respectively). (Table 3.4)

The results show that there was insignificant differente in the mean of platelet count , MPV , PDW , PCT and P-LCR in smoker who smoke more than 10 years and who smoke less than 10 years .(P.Value 0.427 , 0.380 , 0.309 , 0.767 , 0.430). (Table 3.5).

There was no significant different in smoker who smoke less than 10 cigarette per day when compare to smoker who smoke more than 10 cigarette per day in the mean of platelet count , MPV , PDW , PCT and P-LCR (P.value 0.796 , 0.360 , 0.188 , 0.761 , 0.621). (Table 3.6).

Results

Table (3.1) frequency and percentage of case and control among study participant:

Sample	Frequency	Percentage
Case	40	50%
Control	40	50%
Total	80	100%

Table (3.2) the frequency and percent of intensity of smoking:

intensity of smoking cigarette/ day	Frequency	Percentage
10 and less	20	50%
More than 10	20	50%
Total	40	100%

Table (3.3) the frequency and percent of duration of smoking:

Duration group /year	Frequency	Percentage
10 and Less	34	85%
More than 10	6	15%
Total	40	100%

Table (3.4) Comparison of mean of platelet count and platelet indices between case group and control group:

Parameter	Mean ± STD of smokers	Mean ±STD For non-smokers	P.value
Platelet×10/L	226.7 ± 56.7	233.9 ± 54.2	0.560
MPV fl	9.3 ± 0.8	9.5 ± 0.7	0.285
PDW fl	12 ± 2.2	12.2 ± 1.8	0.638
PCT %	0.21 ± 0.04	0.21 ± 0.04	0.325
L-PCR %	9.4 ± 2.20	10.1 ± 2.22	0.470

Chapter Four

Discussion, Conclusion and Recommendation

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4.1 Discussion:

Smoking may contribute to the incidence of atherosclerosis, as well as acute complications, especially thrombosis. It is believed that disturbances of platelet function, especially aggregation, is the essential mechanism responsible for this pathology. However, the effect of smoking on the quantity of platelets might be another contributing factor.

This study aimed to determine the platelet count and platelet indices MPV, PDW, PCT and P-LCR among healthy smokers in Khartoum state.

40 smokers and 40 healthy nonsmoker (control) was participate in the study, age of participant was 18-45 years and most frequent age group was (18-25)years.

The result show that there is no significant different between the smokers and nonsmokers in the platelet count and this study agreed with (Inal, 2014) in Istanbul and (Tulgar, 2016) in Istanbul and disagreed with study in Pakistan (Asif, 2013) and (Varol, 2013) in Spanta city which indicated there was significant decreased in PLT count in smokers than non-smokers. Also result disagree with (Ahmed, 2016) in Rania city-Iraq who found there was significant increase in PLT count among smokers group.

This study shows there was no significant different between the smokers and nonsmokers in the platelet indices MPV, PDW, PCT and P-LCR and this agreed

with a study in Istanbul (Tulgar, 2016) and partial agree with (Ahmed, 2016) study in Iraq which say there was no significant different in MPV, PDW and P-LCR.

And disagree with study in Pakistan (Asif, 2013) that found significant decreased in PCT, and study conduct in Rania city (Ahmed, 2016) show increase in PCT. Also differ from study in Spanta (Varol, 2013) which found a significant increase in MPV among smokers.

According to the result of our study, we conclude that the platelet count and platelet parameters in Sudanese smokers and non-smokers were not different. However, because this study is only a small study, further studies with larger sample size focusing in the details of hemostasis are also recommended.

4.2 Conclusion:

1-Study found that there is no statistical different between the mean of platelet count and platelet indices MPV, PDW, PCT and P-LCR in the smoker and nonsmoker.

4.3 Recommendations:

1-Another study should be conducted with large sample size.

2-Another study should be conducted to evaluate platelet function and other coagulation parameter in smoke

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Appendix

Sudan University of science &technology

Collage of Graduate studies

Assessment of platelet count and platelet indices among Sudanese cigarette smokers In Khartoum state

Questionnaire

Name……………………..   Age…………

Duration of smoking:…………….

Number of sigerate / day:………………

- Exclusion criteria:

1/Have you get blood or blood component trasfusion   Yes…..   No.....

2/ If you have taking any of (Asprin,Warfarin,Heparin) Yes…………………No………………………

3/ If you suffering of any (Hypertantion,DM,)Yes………………..N0…………………….

4/If you taking alcohol.   Yes…………………….No……………….

- Investigation:

Plt count

PDW

MPV

PCT

P-LCR   ***Signatur………………*Date.............................**