Chapter one

Introduction and Literature review

1.1 Introduction:

Smoking is a practice in which a substance, most commonly tobacco, is burned and the smoke istasted or inhaled.[1]

Cigarette is a French wordforsmallcigar,itis a product consumed through smoking and manufactured out of cured and finally cut tobacco leaves and reconstituted tobacco, often combined with other additives, then rolled or stuffed into a paper-wrapped cylinder. [2]

Cigarette smoking was an uncommon behavior earlier in the 19th century has increased tremendously over a period of years. Growing incidence of smoke in young population is still a matter of serious concern for health professionals. [3]

Apart from smoking tobacco had a number of uses as medicine. As a pain killer it was used for earache and toothache and occasionally as a poultice, also if the tobacco was mixed with the leaves of the small desert Sageor the root of Indian Balsam the addition of which was thought to be particularly good for asthma. [4]

Heavy smoking is the commonest cause of ischemic heart disease and death in30-40 years of age group who are likely to be free from myocardial risk factors.[3]

Agents in smoke have a direct irritant effect on the tracheobronchial mucosa, producing inflammation. Atherosclerosis, myocardial infarction, also been strongly linked to cigarette smoking; causal including increased platelet aggregation.[5]

The hyper thrombotic state in smokers may be due to increase in the platelet aggregability and increased platelet activity which initiate clot formation leading to occlusive vascular disease, the most aggregating agent elevated in smokers is epinephrine and nor epinephrine and this due to nicotine induced stimulation of adrenal medulla,epinephrine bind to

specific receptor on platelet, stimulates prostaglandin synthesis from the platelets.[3]

The health effects of smoking includes; wrinkling of the skin, bad breath, smelling cloths and hair, yellowish fingers and finger nail, increased risk of macular degeneration (cause blindness in the elderly due to vascular degeneration, stained teeth, tobacco associated with chronic obstructive pulmonary disease, increased airway reactivity, and increased frequency of pulmonary infection.[6]

Some chemical components of cigarette smoking:

Nicotine: is a powerful insecticide poisonous for the nervous systems. Furthermore, there is enough (50mg) in four cigarettes to kill a man in just a few minutes if it were injected directly into the blood stream. Nicotine causes accelerated heart rate, also increases the consumption of lipids.[7]

Carbone monoxide (CO): this is asphyxiating gas produced by cars which makes up 1.5% of exhaust Fumes. But smokers inhaling cigarette smoke breathe in 3.2% carbon monoxide. When we smoke the CO attaches itself to hemoglobin 203 times more quickly then oxygen dose there by displacing the oxygen, this in turn asphyxiates the organism this cause the following: narrowing of the arteries, blood clots, arteritis, gangrene, but also visual and mental problems. [7]

Irritants: these substances paralyze and then destroy the cilia of the bronchial tubes, responsible for filtering and cleaning the lung. The slowdown respiratory output irritates the mucus membranes, causing cough, infections.[7]

Tars: as the cilia are blocked the tars in cigarette smoke are deposited and collect on the walls of the respiratory tract and lungs to cause them to turn black. The tar is responsible for 95% of lung cancer. By smoke one packet of cigarettes every day, smoker is pouring a cupful of these tars into his or her lungs every year (225grams on average).[7]

1.2 Literature review:

1.2.1 Platelet:

Platelets were recognized in 1882 by Bizzozero as a cellstructure different from red and white cells. However, itwas not until 1970 that platelets relationship to hemostasisand thrombosis became so important. There is no reserve of platelet in bone marrow: 80% are in circulation and20% are in the red pulp of spleen. [8]

1.2.1.1 Platelet production:

Platelets are produced in bone marrow by fragmentation of the cytoplasm of megakaryocyteswhich develop from pluripotential stem cell (Fig 1-1)that has been influenced by colony stimulating factor (CSF).[9,10]

Thromopoietin (TPO) is the major regulator of plateletproduction and is constitutively produced by theliver and kidneys.[11]

Thromopoietin activity results from different cytokines: IL-3 and granulocyte macrophage stimulating factor [GM-CFU]. These substances have been shown to be able to increase megakaryocytic size, maturational stage and ploidy. [12]

Thrombopoiesis is process by which thrombocyte (platelet) formed in bone marrowthrough a phase characterized by mitotic division of a progenitor cells followed by a wave of nuclear endoredupliction which is a process in which DNA and other event of mitosis occur without subsequent division of the cytoplasmic membrane into identical daughter.[13]

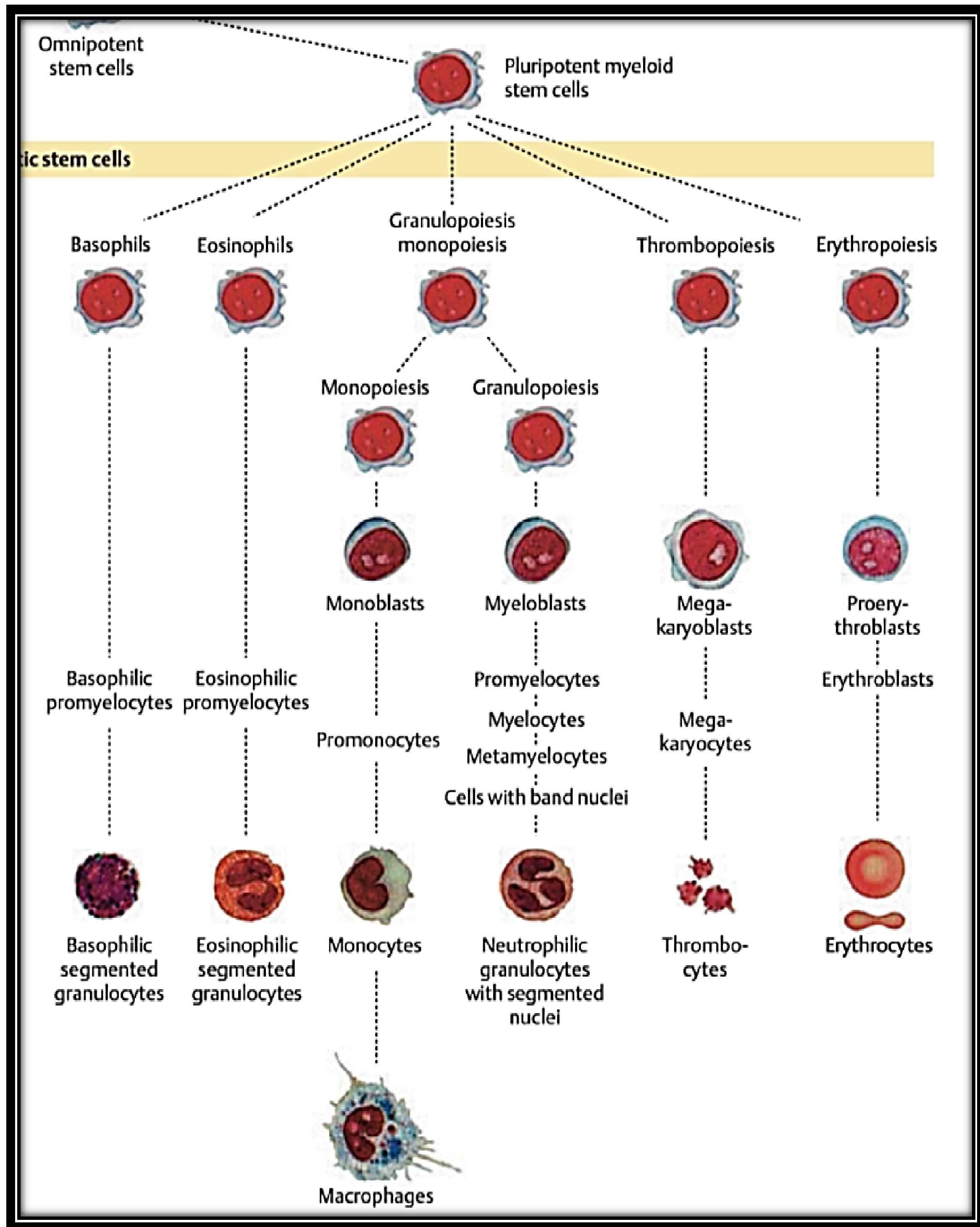

Figure 1-1: development of megakaryocyte from pluripotential stem cell.[14]

1.2.1.1.1 The megakaryocytic cell series: Is defined as serial maturation of megakaryoblast for production of platelet in bone marrow (Fig.1-2). [9]

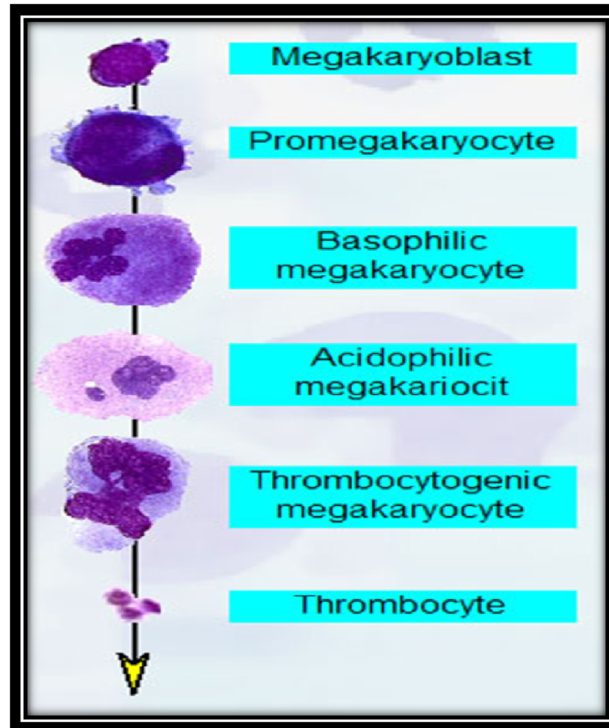

Figure 1-2:Megakaryocytic series. [15]

1.2.1.1.1.1 Megakaryoblasts [MK1]: Are the first cells in maturation sequence which are typically 10-15 micron in diameter with a higher nuclear matter to cytoplasmratio; they have a single nucleus with the 2-6 nucleoli. The cytoplasm is scantly, blue and contain no granules. [10]

1.2.1.1.1.2 Promegakaryocyte [MK2]: megakaryoblast mature into a promegakaryocyte which enlarges to80 micron (25-100micron) [10]and large than its precursor because it has undergone endoredupliction, such replication leads ultimately to the formation of very large cells containing up to32 times the normal diploid content of DNA. The chromatin is more deeply basophilic than in the megakaryoblast, and has deeply basophilic cytoplasm containing some granules.[16]

1.2.1.1.1.3 Basophilic megakaryocytes [MK 3]: Distinct granulation and final division of nucleus occur. Cytoplasmic lines of demarcation begin to be evident out liting individual cytoplasmic fragment later to be released as platelets .[10]

1.2.1.1.1.4 Megakaryocyte [MK4]: are large cells (80-150 micron), which undergo endomitosis(nuclear replication without cellular division) or creates multi-lobed nucleus to increase the DNA content to enable the cell to expand its protein synthesis capacity to generate 2000–3000 platelets per megakaryocyte.[8,17]

The distinctive feature of megakaryocyte is that it is multilobular not multinucleated.[12]

The mature megakaryocyte release cytoplasmic fragments in process called budding or shedding of platelets . [10]

The time interval from differentiation of the human stem cell to production of platelet approximately 10 days. [9]

1.2.1.1.1.5 Platelets: Are the small, a nucleated, terminal stage of development of megakaryocytic series. They are discoid and have a diameter of 1-4 micron. The cytoplasm stain light blue and contain small red-purple granules.[16]

The normal platelet count is about 250×10^3 /mm^3 (range150-400×10^3/mm^3)with a mean volume 7-11 fland normal platelet life span is 7-10 days.[11]

1.2.1.2 Platelet structure:

Examination of platelet with an electron microscope reveals a variety of structures (fig1.3) these structures are fundamental to the functioning of the Platelet.[12]

platelet structure is classified into four general areas: the platelet surface, membranous structures, cytoskeleton (sol-gel zone), and granules.[18]

1.2.1.2.1 Platelet surface:

Plasma Membrane: is a bilayer of phospholipids in which cholesterol, glycolipids and glycoproteins (GPs)are embedded. [19]

Glycoproteins such as GPIa for the adherence of platelet to charged collagen, GPIb-IX-V complex is essential to mediate the initial adhesion of platelets to immobilized

vWFexposed at sites of vascular injury, GP IIbIIIais the receptor for fibrinogen, GPV is produced by liver and absorbed from plasma to in to the platelet alpha granules.[20]

Glycocalyx: is a fuzzy layer of lipids, sugars, and proteins, coats the outside surface of the platelet plasma membrane which adsorbed plasma proteins and produces a net negative surface charge mainly as a result of sialic acid residues on certain proteins such as GPIb. [18]

Platelet ABO and HLA antigens are expressed in the glycocalyx region. [10]

1.2.1.2.2 Platelet Membranous Systems:

Two membranous systems of platelets; the Surface-Connected Canalicular System and the dense tubular system. [18]

Surface-Connected Canalicular System (SCCS): also called the open canalicular system (OCS), which is composed of invaginations of the plasma membrane, it connect the internal contents with the surface. [18, 19]

Dense Tubules (DTS): is a closed-channel system consisting of narrow, membrane-limited tubules. In fact, residual smooth endoplasmic reticulum from the megakaryocyte. This channel system is involved in the regulation of intracellular calcium transport; it is also the site of prostaglandin (PG) synthesis in platelets. [18]

1.2.1.2.3 Platelet Cytoskeleton:

The discoid shape of the platelet is formed by the platelet cytoskeleton, which consists of a spectrin-based membrane skeleton; circumferential bands of single microtubule that lies beneath the plasma membrane; and a rigid actin filament network that fills the cytoplasm of the cell. [17]

Microfilaments: The platelet is rich in actin, a protein that can polymerize into micro filamentous bundles, when platelets change shape, the filopodia they form contain bundles of microfilaments. [21]

Microtubules: A circumferential microtubule band that supports the discoid form of the platelet is made of tubulin. Platelet activation results in microtubule disassembly and then reassembly; such alterations in the marginal microtubule bundle result in platelet shape changes. [18]

1.2.1.2.4 Platelet Granules and Organelles:

Alpha (α) granules: contain β–thromboglobulin (β TG) and platelet factor 4(PF4), fibrinogen, factor V, factor XIII, von Willebrand factor (vWF), platelet derived growth factor (PDGF), protease inhibitors. [19]

Dense bodies: named because their appearance when viewed by electron microscopy, also called storage granules because of their functional characteristics. Each platelet has up to 10 dense granules, contain adenine triphosphate (ATP), adenine diphosphate (ADP), ionized calcium, magnesium, epinephrine, phosphate and serotonin (5HT). [11, 12, 17]

Lysosomes: contain hydrolytic enzymes, microbiocidal enzymes. [12]

Organelles: Glycogen and mitochondria. [18]

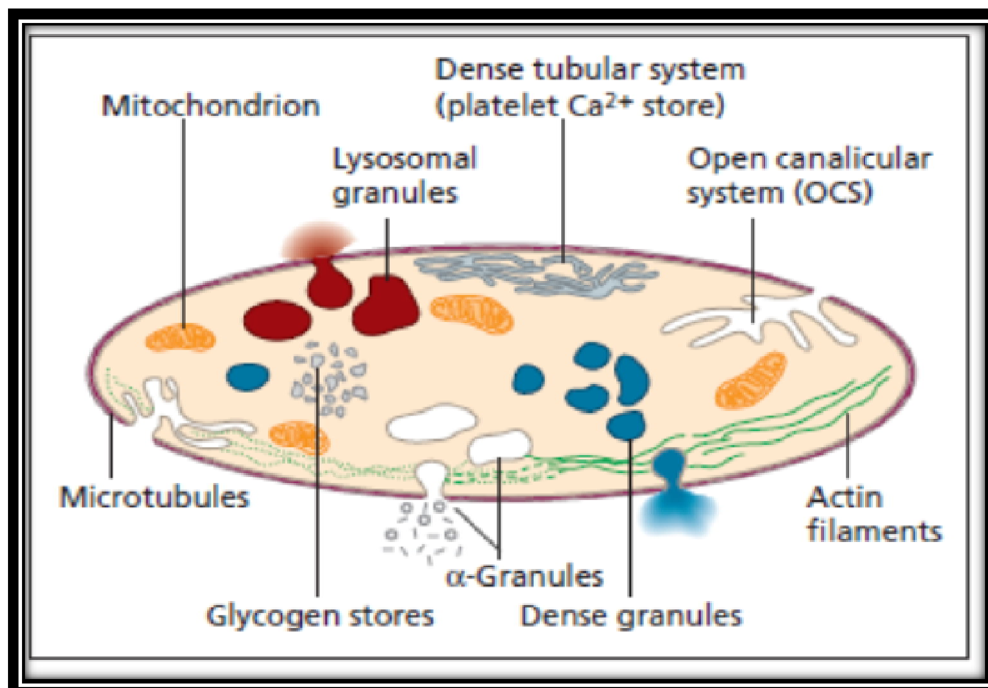

Figure 1-3: Plateletultra-structures. [17]

1.2.1.3 Platelet function:

The main function of platelet is the formation of mechanical plug during the normal hemostatic response to vascular injury. [11]

Platelet also maintain vascular integrity by preventing red blood cells from escaping from blood vessel into tissue.Activated platelets play an important role in inflammation and express or release a number of molecules that lead to leukocyte activation. [20]

After initial injury there is brief period of arterial vasoconstriction by secretion of endothelin also epinephrine and serotonin promote vasoconstriction. [12]

1.2.1.3.1 Platelet adhesion: If vascular injury exposes the endothelial surface and underlying collagen, platelet adhesion to sub endothelial connective tissue especially collagen (fig.1.4).[12]

Platelet adhesion to extracellular matrix (ECM) is mediated largely via interaction with vWF,which acts as a bridge between platelet surface receptor (GPIb) and exposed collagen, which result in an initial monolayer of adhering platelets.[22]

In large vessels platelet adhesion is independent on vWF and involve direct adhesion of platelets to collagen fibrils via GPIIb/IIIa complex and GPIa. [16]

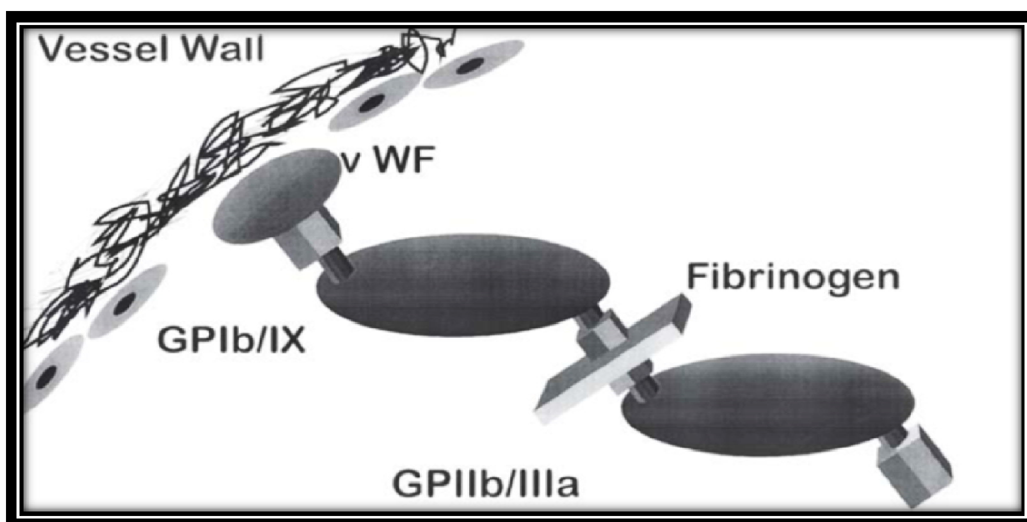

Figure.1.4 platelet adhesion and aggregation. [23]

1.2.1.3.2 Shape change and spreading: Onceactivated platelets immediately change shape from disc to a tiny sphere with numerous projecting pseudopods.[22]

Platelet extends pseudopodia to enable them to attach to other platelets and to the vessel wall.[17]

1.2.1.3.3 Platelet release reaction: Secretion of the contents of both types of granules occurs soon after adhesion. The process is initiated by binding of agonist to platelet receptors followed by intracellular phosphorylation cascade.[5]

1.2.1.3.4 Platelet aggregation: Is used to describe cross-linking of platelets through binding of fibrinogen (figure1.4) or other bivalent or multivalent ligands such as vWF to the integrin (GpIIb-IIIa) on adjacent cells. [17]

Follow adhesion and secretion besides ADP, vasoconstrictor TXA2 secreted by platelets is also stimulus for platelet aggregation.[22]

Bridge formed by fibrinogen in the presence of calcium produce a sticky surface of platelets this results in aggregation.[12]

Platelet aggregation may occur by two independent but closely linked pathways. The first pathway involves arachidonic acid metabolism is the activation of phospholipase release free arachidonic acid from the membrane phospholipids. About 50% of free arachidonic acid is converted by a lipooxygenase to a series of products include leukotriene which are chemotactants of white cells. The remaining 50% of arachidonic acid is converted by cyclooxygenase into labile cyclicendoperoxidase, most of which are in turn converted by thromboxane synthetase into TXA2, which causing further local platelet aggregation. It executes these effects by raising intracellular cytoplasmic free calcium concentration and binding to specify granule receptors. [17]

The second pathway can proceed completely independently from the first one: various platelet agonists, including thrombin and collagen, produce a brisk increase in the free cytoplasmic calcium to cause the release reaction. Calcium

is released from dense tubular system to form complex with calmodulin; this complex and free calcium act as co-enzyme for the release reaction, for the regulatory proteins, as well as of actin myosin and contractile system, also for generation of TXA2.[17]

1.2.1.3.5 Clot formation and retraction: The highly localized enhancement of ongoing platelet activation by ADP and TXA2 result in platelet plug which large enough to plug the area of endothelial injury. In this platelet plug the platelets are completely degenerated and adherent to each other, this is followed by clot retraction which is mediated by GPIIb/Illa receptors which link the cytoplasmic actin filaments to the surface bound fibrin polymers. [11]

1.2.1.3.6 Procoagulant activity of platelet:

After platelet aggregation and release, the exposed membrane phospholipid (platelet factor 3) is available for two reactions in the coagulation cascade. Both phospholipid-mediated reactions are calcium ion dependent. The first (tenase) involves factors IXa, VIlla and X in the formation of factor Xa. The second (prothrombinase) results in the formation of thrombin from the interaction of factors Xa, Va and prothrombin (II). The phospholipid surface forms an ideal template for the crucial concentration and orientation of these proteins. [11]

1.2.1.3.7 Protease inhibitors: All known plasma protease inhibitors are also localized to platelet α-granules. These include α_1-protease inhibitor, α_2-macroglobulin, $\alpha_2$antiplasmin, plasminogen activator inhibitor-1, protease nexin I (thrombin inhibitor), and protease nexin II (factors IXa and XIa inhibitors). [18]

1.2.1.3.8 Growth factor: PDGF found in the specific granules of platelets stimulates vascular smooth muscle cells to multiply and thus may hasten vascular healing following injury. [11]

1.2.1.4 Platelet natural inhibition: Ifplatelets are activated they are inhibited from adhering to surrounding uninjured endothelium by endothelial prostacyclin [PGI_2] and nitric oxide both are vasoconstrictor and inhibitor of platelet aggregation. [5]

1.2.1.5 Platelet indices: Circulatory platelets are very different in size, metabolism and functional activity. The largest are more reactive and produce a great quantity of thrombogenic factor.[24,25]

The platelet parameter which includes the platelet distribution width, mean platelet volume, platelet large cell ratio and Plateletcrit,have been available in the laboratory routine using blood cell counters.[26]

When blood comes in contact with EDTA, platelet rapidly change shape from disk with diameters of 2 to 4 micron to spheroids covered with filamentous extension. The platelet spherical transformation is initially iso-volumetric, but within 1to2 hours, the volume progressively changes to reach an equilibrium condition. [27]

1.2.1.5.1 Mean platelet volume (MPV): Is a measure of the average volume of platelet in a sample. It is analogous the erythrocytic MCV.[12]

Platelet size is a difficult to accurately quantitate and use diagnostically, because of a wide physiologic variation of the MPV in normal subjects and susceptibility of anticoagulated platelets to time-dependent swelling in vitro.[21]

In healthy, there is inverse relationship between platelet count and size; the volume increase as platelet count decrease. Patient with a lower platelet count normally have a higher MPV. [12]

Large platelet characterized by higher MPV– contains more dense granules, metabolically and enzymatically more active than small platelet; therefore these platelets have higher thrombotic potential. [28]

Established cardiovascular risk factors, such as smoking, hypertension, dyslipidemia and diabetes can influenced MPV, and evidence derived from prospective studies, suggest correlation between increased MPV and the risk of thrombosis. [29]

MPV is calculated as following formula: MPV [fl] = $\frac{PCT\% \times 1000}{PLT(\frac{1000}{ml})}$

Where PCT % represent the value weighted with platelet frequency and is called Plateletcrit or platelet volume ration. It is calculated by platelet plus height detection method. [30]

Elevated MPV values have been reported in subjects with type 2 diabetes, particularly in subjects with vascular complications. [31]

The automated blood count shows an increased MPV and PDW when there is increased platelet consumption or destruction and a low MPV when there is failure of bone marrow output. [32]

The MPV is often decreased in a plastic anemia, megaloblastic anemia, after chemotherapy,the MPV rising as platelet count falls platelet destruction with DIC causes. TheMPVincreases in patients with myeloproliferative disorders or heterozygous thalassemia, Idiopathic thrombocytopenic purpura, sickle cell anemia have been investigated in connection with both thrombosis and inflammation. [29]

1.2.1.5.2 Platelet distribution width(PDW): Is a measure of uniformity of platelet size in a blood specimen.[12]

Platelet activation causes morphologic changes of platelets, including both the spherical shape and pseudopodia, platelet with increased number and size of pseudopodia differ in sizes, possibly effecting PDW, which is a more specific marker of platelet activation, since it does not increase during simple platelet swelling.[33]

The PDW has been found to be of some use in distinguishing essential thrombocythaemia (PDW increased) from reactive thrombocytosis (PDW normal).[22]

1.2.1.5.3 Platelet large cell ratio (P-LCR): It is representing the proportion of large platelet (those > 12fl) to the total platelet count and may be an indicator of possible platelet clumping, giant platelets or cell fragment.[10]

P-LCR, reported by some instrument as the number of platelets falling above the 12 fl threshold on the platelet size histogram divided by the total number of platelets.[22]

In patients with high platelet counts, P-LCR was significantly decreased in reactive thrombocytosis than in neoplastic thrombocytosis. P-LCR was increased in destructive thrombocytopenia than those with hypo proliferative thrombocytopenia, [34]

Increased percentage of large platelets also havebeen observed in patients with hyperlipidemia and this has been suggested as a possible risk factor for thrombosis. [32]

1.2.1.5.4 Plateletcrit (PCT): Is a measure of total platelet mass. PCT which could screen thrombocytopenia, thrombocytosis and normal platelet count. Platelets with normal platelet count have a PCT within the normal range of 0.20-0.36%. [35]

Table (1.1) Normal values of platelet indices: [30]

	MPV(fl)	PDW(fl)	P-LCR (%)
Male	8.1-12.4	9.8-18.0	10.7-45.0
Female	8.5-12.4	9.4-18.1	14.3-44.0

1.2.1.6 Platelet disorders:

Can be classified as quantitative (thrombocytopenia and thrombocytosis) or qualitative (thrombocytopathy). [12]

1.2.1.6.1 Quantitative platelet disorders:

1.2.1.6.1.1 Thrombocytopenia:

Is defined as reduction in the periphery blood platelet count below the lower normal limit of $150X10^9$/L. [16]

Spontaneous bleeding usually occurs with platelet counts less than 20,000/µl. Thrombocytopenia is due either to decreased bone marrow production or increased destruction of the platelets from the circulation, or both. [23]

1.2.1.6.1.1.1 Failure of platelet production:

Wiskott-Aldrich syndrome**:** Is an X-linked disorder with thrombocytopenia, eczema, immune deficiency, and the platelets are small and functionally abnormal.[36]

May Hegglin anomaly**:** Includes thrombocytopenia with giant bizarre platelets and inclusions in the white cells. It is inherited as autosomal dominant. [37]

1.2.1.6.1.1.2 Increased destruction of platelets:

Autoimmune (idiopathic) thrombocytopenic purpura:refers to cases of thrombocytopenia with no apparent etiology or underlying disease states.[10]May be divided into chronic and acute forms.[11]

Chronic idiopathic thrombocytopenic purpura: Is the most common cause of thrombocytopenia without anemia or neutropenia. It is usually idiopathic but may be seen in association with other diseases such as systemic lupus erythematous (SLE), human immunodeficiency virus (HIV) infection, chronic lymphocytic leukemia (CLL) or autoimmune hemolytic anemia.[11]

Acute idiopathic thrombocytopenic purpura: This is most common in childrenand occur after immunization with live vaccinefor measles, chickenpox, mumps and smallpox. [10]

Post-transfusionpurpura: Thrombocytopenia occurring approximately 10 days after a blood transfusion has been attributed to antibodies in the recipient developing against the human platelet antigen-la (HPA-la) (absent from the patient's own platelets) on transfused platelets. [11]

Drug-induced immune thrombocytopenia: An immunological mechanism has been demonstrated as the cause of many drug-induced thrombocytopenia includeheparinwhich are particularly common cause. the platelet count is often less than 10 x 10^9/L, the bone marrow shows normal or increased numbers of megakaryocyte.[11]

Approximately 20 to 30% of patients given heparin develop thrombocytopenia, so it is the most often drug causes thrombocytopenia in hospitalized patients. [37]

Thrombotic thrombocytopenic purpura (TTP): the presenting features can be fever, renal impairment, and intravascular hemolysis, resulting in thrombocytopenia. The condition is caused by an autoantibody to a protease enzyme which is responsible for cleaving the ultra-high molecular weight multimers of von Willebrand factor. The development of this antibody causes a circulating excess of highly active multimers, causing intravascular platelet agglutination in vivo. The condition is suspected clinically by thrombocytopenia, red cell fragmentation on the blood film, and a reticulocytosis. [36]

Infections: It seems likely that the thrombocytopenia associated with many viral and protozoan infections is immune-mediated. In HIV infection, reduced platelet production is also involved.[11]

Massive transfusion syndrome: Platelets are unstable in blood stored at 4°C and the platelet count rapidly falls in blood stored for more than 24 h. Patients transfused with massive amounts of stored blood (more than 10 units over a 24h period) frequently show abnormal dotting and thrombocytopenia. These should be corrected by the use of platelet transfusions and FFP.[11]

Disseminated intravascular coagulation: Thrombocytopenia may result from an increased rate of platelet destruction through consumption of platelets because of their participation in DIC. [11]

1.2.1.6.1.2 Thrombocytosis: Is defined as a platelet count exceeding the upper limit of the reference range (value of > 400,000 platelets/L).The term thrombocythaemia is sometimes used synonymously with thrombocytosis, but it should be restricted to the chronic myeloproliferative disorder primary thrombocythaemia (essential thrombocythaemia). The cause for an increased platelet count may be primary or secondary.[37]

Primary thrombocytosis: is seen in the myeloproliferative disorders in which case platelets are high in number but have an impaired functionand may be associated with thrombosis or bleeding.[37]

Reactive thrombocytosis: is usually secondary to some other condition and is not associated with an increased risk of thrombosis.Secondary causes of thrombocytosis include infection, inflammation, malignancy, and iron deficiency anemia. [37]

1.2.1.6.2. Qualitative platelet disorders:

Qualitative defects may result in excessive bleeding, even though the platelet count is normal. [16]

Platelets have a complex ultra-structure comprising a multitude of molecules and the malfunctioning of any of these may give rise to a specific disease.[11]

1.2.1.6.2.1 Congenital qualitative platelet defects:

Thrombasthenia (Glanzmann's disease): This autosomal recessive disorder leads to failure of primary platelet aggregation because of a deficiency of membrane GPIIb. It usually presents in the neonatal period and, characteristically, platelets fail to aggregate in vitro to any agonist except ristocetin.[11]

Bernard-Souliersyndrome (BSS): There is thrombocytopenia and the platelets are larger than normal and there is a deficiency of GPIb. There is defective binding to vWF, defective adherence to exposed sub endothelial connective tissues and platelets do not aggregate with ristocetin. [11]

Granules defect:

Storage pool disease (δ-SPD); autosomal dominant, platelet aggregation with ADP and collagen is defective, but to arachidonic acid is normal. Cause mild to moderate bleeding tendency similar to that induced by aspirin. [16]

Gray platelet syndrome; is an extremely rare autosomal dominant disorder with a mild bleeding diathesis. The characteristics are prolongation of skin bleeding time, thrombocytopenia, and enlarged platelets with an unusual gray color on routine peripheral blood film. [11]

Combined defects (αδ-SPD): Deficiencies in both major platelets granules, additional abnormalities in the metabolism of the platelets have been reported. The group of inherited disorders: in which δ storage pool deficiency is one of a

number of defects consists of Hermansky-Puudlack syndrome (bleeding tendency, and pigmented macrophage in the bone marrow), Wiskott–Aldrich syndrome, and Chediak-Higashi syndrome (susceptibility to infection, thrombocytopenia, and defective pigmentation of skin and retina).[11]

1.2.1.6.2.2 Acquired qualitative platelet dysfunction:

Antiplatelet drugs: Aspirin therapy is the most common cause of defective platelet function. The cause of the aspirin defect is inhibition of cyclooxygenasewith impaired thromboxane A_2 synthesis. Clopidogrel inhibits binding of ADP to its platelet receptor.[11]

Hyperglobulinaemia: Associated with multiple myeloma or Waldenstrom's disease may cause interference with platelet adherence, release and aggregation.[11]

Myeloproliferative and myelodysplastic disorders: Intrinsic abnormalities of platelet function occur in many patients with essential thrombocythaemia and other myeloproliferative and myelodysplastic diseases. [11]

Thrombocytopenia frequently occurs in patients with underlying malignancies who have received chemotherapy or radiotherapy that destroy the hematological stem cells. [23]

Drug induced platelet abnormalities: Many drugs affect platelet function in vitro. Aspirin ingestion results in abnormal platelet function with a moderately prolonged bleeding time and a defective platelet aggregation In vitro, the penicillin impair the interaction of the VWF and aggregation agonists with the platelet surface membraneof platelet function comparable to those observed in dysproteinemias.[3]

1.3 Previous studies:

The result of study conducted in Niger by (Aghaji M *et al.*1990) involved 176 smokers and 176 non-smokers to determine the relationship of white cells and platelet count in cigarette smoking. Showed that platelet count was higher for regular Nigerian smoker than for non-smoker was statistically significant ($t=2.64$ $p_{=}$ 0.0046). [38]

The result of cross sectional study conducted in Thai police in Bangkok by (Sirwansaksri J *et al.*2004) to compare platelet count and parameters in 25 smokers and 5 non-smokers. Showed that platelet count and parameters of the subjects were not significantly different. [39]

In 2013, Varol E *et al* studied the value of MPV in 116regular smokers (57 females & 59 males) and 90 healthy non-smokers (49 females and 41 males) The MPV values were significantly higher in smokers than non-smokers (8.8 ±0.9 vs. 8.0 ±0.8 fl, respectively; $p< 0.001$). [40]

Gitte RN *et al* in 2011, studied the effect of cigarette smoking on plasma fibrinogen and platelet count in 125 healthy smokers and 125 healthy non-smokers in India, the platelet count was done by using Beckman Coulter Automatic Analyzer; AcT5diffCP. The mean platelet count for smokers was 257325 per mm^3 and for non-smokers was 215483.3 per mm^3. Platelet count of smokers and non-smokers was statistically significant ($p < 0.0001$). [41]

The result of study conducted by Parlak A and Kilci H involved 60 smokers and 55 non-smokers to determine the relation between platelet parameters and smoking status. Showed that platelet count were not significantly different between groups. MPV levels were higher in smokers than non-smokers (7.80 ± 0.90 vs. 7.57 ± 0.74 respectively, $p > 0.05$) but statistically not significant. PDW levels were also high in smokers compared non-smokers (13.98 ± 1.49 vs. 13.51 ± 1.19 respectively, $p > 0.05$) but not significantly different. [42]

The result conducted by Arslan E *et al* in 2008, included 56 smokers and 46 non-smokers to study the effect of smoking on MPV and lipid profile in young

male subjects. Showed that no significant difference was found for MPV between the groups smoking (8.57±0.8 fl) non-smoking (8.67±0.8 fl, P =0.66).[43]

The result of study conducted by Butkiewicz AM. *et al* to study if the smoking affects Thrombopoiesis and platelet activation in women and men. Showed that lower platelet count in smokers than non-smokers (237±39.52 vs. 258±40.81×10^9/L, P=0.0002). [44]

1.4 Rationale:

Cigarette smoking is one of the major life style factors affecting the health and the incidence is still increased. Cigarette smoking is considered as a risk factor for hyper thrombotic state and we want to know the effect of smoking on platelet count and its parameters because many studies have been included to study the effect of smoking on platelet count but different results were obtained.Also to highlight the importance and role of platelet indices in diagnosis platelet disorders.

1.5Objectives:

1.5.1 General objectives:

To measure the platelet count and platelet indices among cigarette smokers in Khartoum state.

1.5.2 Specific objectives:

1-To estimate platelet count and indices in smokers and non-smokers.

2- To compare the platelet parameters (MPV, PDW, P-LCR, PCT and platelets count) among cigarette smokers and non-smokers.

3- To assess platelet count and indices according to number of cigarette per day among cigarette smokers.

4- To compare between platelet count and indices in cigarette smokers according to smoking duration.

Chapter two

Materials and Methods

2.1 Study design:

This is analytical cross sectional study, conducted in Khartoum state in the period from December 2013 to January 2014.

2.2 Study population:

One hundred and seventy individual were participated in this study and classified into two groups: 100 individual were cigarette smokers, and 70 individual were non cigarette smokers.

2.2.1 Inclusion criteria:

Case group were smoke at least 5 cigarettes per day for a minimum period of one year. And must not be smoking any other kinds of smoking (as Shisha).

2.2.2 Exclusion criteria:

Previous history of thrombosis, hypertension and or diabetes mellitus.

2.3 Ethical consideration:

The specimens and information that collected from the participants were under privacy and confidentially. The aim of the research was explain for the subjects under the study in simple language and they understood the research idea.

2.4 Data collection: The data was collected by the direct interview through designed questionnaire which include the age, number of cigarettes per day, duration of smoking, and excluded diseases as diabetes and hypertension.

2.5 Data analysis:

The data was computed and analyzed to obtain the platelet count and indices using statistic package for social science (SPSS) program version 11.5. The statistical analysis was performed by using Independent sample T test.

2.6 Methods:

2.6.1 Method of blood sample collection:

2.6.1.1 Requirements for blood collection:

Ethylene diamine tetra acetic acid (EDTA.K3) containers (2.5 ml Marina pharma).

Cotton.

Alcohol (70%).

Syringe and tourniquet.

2.6.1.2 Procedure:

The person was sitting on table then the arm was positioned on the armrest so that the vein identified and after that the skin was cleaned with 70% alcohol and allowed to dry. Tourniquet was applied to the arm, sufficiently tightly to distend the vein, but not so tightly that discomfort may arise, the personal details was checked up on the forearms and blood vials, 2.5ml of blood sample was collected by syringe. Then blood sample was collected in EDTA.K3 (1.2mg/ml blood) anticoagulant. EDTA blood sample was analyzed by sysmex KX-21N (semi-automated hematological analyzer). [45]

2.6.2 Sysmex KX-21N:

2.6.2.1 Principle of KX-21N:

This instrument perform blood count to measure WBC and differential counts RBCSs,HCT,MCV,MCH,MCHC,Hb and platelet count. Blood sample is aspirated, measured to predetermine, andthen diluted at specified ratio. Then fed into each transducer. Transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes, between which flow direct current blood cells suspended in diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As a direct current resistance change the blood cell count is calculated by counting the pulses, andhistogram of blood cell sizes is blotted by various analysis data. Hemoglobin is measured by noncyanide hemoglobin analysis methods which

rapidly converts blood hemoglobin as oxyhemoglobin and contain no poisonous substance making it suitable for automated methods.[46]

2.6.2.2 Reagents and materials:

Commercial close system reagents were provided by sysmex KX-21N operators and consist of: cell pack and stromatolyser: diluents and lysing reagent for use sysmex. Detergent and cell cleaner: use for cleaning solution to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of sysmex automated analyzer. [46]

2.6.2.3 Procedure of KX-21N:

Whole blood mode:

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

1- 4microliter of blood measured by the sample rotor valve is diluted into 1:500 with 1.996microliter of diluents and brought to the mixing chamber as diluted sample (first step dilution).

2- Out of the 1:500 dilution sample 40 microliter is measured by the sample rotor valve, diluted into 1:25000 with 1.96 microliter of diluents then transferred to the RBC/platelet transducer chamber (second step dilution). 250 microliter of sample in RBC/platelet transducer chamber is aspirated through the aperture. At this time RBCs and platelet are counted by the DC detection method. [30]

2.6.3 Blood film preparation:

Not all automated counters are able detect very small platelets or very large platelets, resulting in underestimation of the platelet count. This emphasizes the importance of examining the blood film. [47]

2.6.3.1 Requirements:

Clean dry slide.

Alcohol.

Leishman's stain.

Pasteur pipette.

Capillary tubes.

2.6.3.2 Technique of making blood film:

In clean and dry slide place a small drop of blood in the centre line of a slide about 1 cm from one end. Then, without delay, place a spreader in front of the drop at an angle of about 30 to the slide and move it back to make contact with the drop. The drop should spread out quickly along the line of contact. With a steady movement of the hand, spread the drop of blood along the slide. The spreader must not be lifted off until the last trace of blood has been spread out; with a correctly sized drop, the film should be about 3 cm in length. It is important that the film of blood finishes at least 1 cm before the end of the slide.[22]

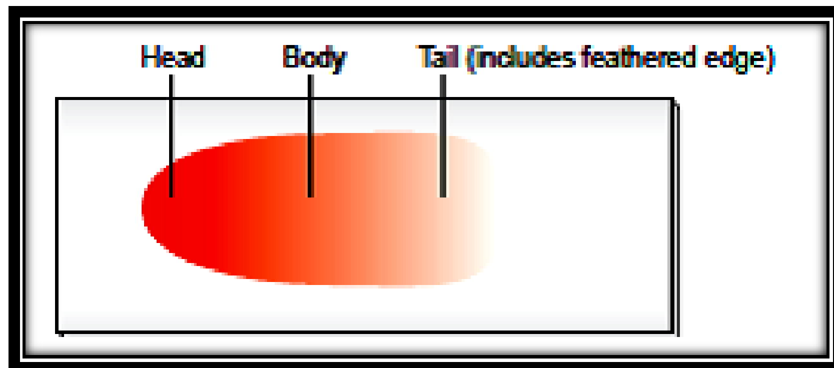

Figure 2-1: Ideal blood film. [22]

2.6.4 Blood film staining:

2.6.4.1 Principle of Leishman's stain:

Leishman's stain is one of Romanoskystains contain eosin Y which is an acidic anionic dye and azure B and other thiazine dyes which are basic cationic dyes. When diluted in buffered water, ionization occurs. Eosin stains the basic components of blood cells pink-red, Azure B and other methylene blue derived dye stain the acidic components of cells various shades of mauve-purple and violet. [48]

2.6.4.2 Preparation of Leishman's stain:

Weigh out 0.2 g of the powdered dye, and transfer it to a conical flask of 200–250 ml capacity. Add 100 ml of methanol and warm the mixture to 50°C for 15 min, occasionally shaking it. Allow the flask to cool and filter the solution. It is then ready for use.[22]

2.6.4.3 Procedure of staining blood film:

Air dry the film and flood the slide with the stain. After 2 min, add double the volume of water and stain the film for 5–7 min. Then wash it in a stream of buffered water until it has acquired a pinkish tinge (up to 2 min). After the back of the slide has been wiped clean, set it up right to dry. [22]

2.6.4.4 Morphology of platelet under light microscopy:

On Romanwosky stained peripheral blood smear, platelets appears as small, round, anuclear cells with prominent reddish –purple granules.[49]

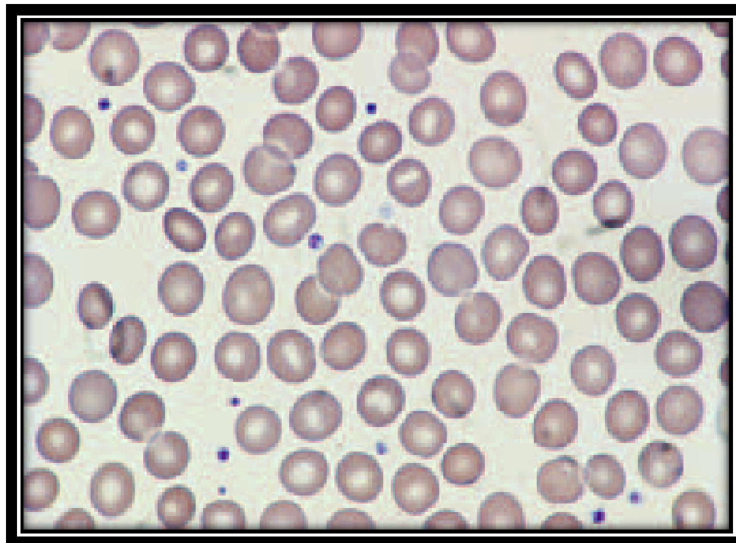

Figure 2.2: Blood film showing normal plateletmorphology. [32]

Chapter three

3.1 Results:

One hundred seventy (170) EDTA venous blood samples were collected from the participants;one hundred are smoker whereas seventy are non-smokers. Platelet count and indices were measured by sysmex KX-21N.

Table (3-1) showed that mean platelet count in smokers was 236.92 ± 50.114 $\times 10^3$ per mm^3) whereas in non-smokers it was 267.13± 54.538$\times 10^3$ per mm^3, with P value =0.000) indicating that there is a significant decrease in platelet count among cigarette smokers.

Table (3-2) showed that the mean platelet volume (MPV) levels were lower in smokers than non-smokers (9.838 ± 0.8568 vs. 10.007 ± 0.9124flrespectively, P value=0.219) but statistically not significant.

Table (3-3) demonstrate that the platelet distribution width (PDW) level was not significantly different in smokers compared with non-smokers (12.035 ± 1.5473 vs.12.0376± 1.7114 fl respectively, P value= 0.178).

Table (3-4) Platelet large cell ratio (PLCR) levels were also lower in smokers than non-smokers (24.172 ± 6.0489 vs. 25.223 ± 6.9511% respectively, P value= 0.296) but statistically not significant.

Table (3-5) show that smoker who smoke <20 cigarette per day was (238.3 ± 45.233) and who smoke ≥ 20 cigarette per day (235.3±55.771) with
P value = 0.768, statistically not significant.

Table(3-6) shows the platelet count in smoker who smoke from less than10 years was 238 $\times 10^3$ ±51.798cell/μl and who smoke more than 10 years was 231$\times 10^3$ ± 43.472cell/μl, p-value=0.584, no significant differences.

Table (3-1): Compare platelet count in cigarette smokers and non-smokers.

Groups	Mean ($\times 10^3$ cell/µl) (N=170)	Std. Deviation	P-value
Smokers (100)	236.92	50.114	0.000
Non-smokers (70)	267.13	54.538	

Table (3-2): Compare mean platelet volume in cigarette smokers and non-smokers.

Groups	Mean(fl) (N=170)	Std. Deviation	P-value
Smokers (100)	9.838	0.8568	0.219
Non-smokers (70)	10.007	0.9124	

Table (3-3): Compare platelet distribution width in cigarette smokers and non-smokers.

Groups	Mean(fl) (N=170)	Std. Deviation	P-value
Smokers (100)	12.035	1.5473	0.178
Non-smokers (70)	12.0376	1.7114	

Table (3-4): Compare platelet large cell ratio in cigarette smokers and non-smokers.

Groups	Mean (%) (N=170)	Std. Deviation	P-value
Smokers (100)	24.172	6.0489	0.296
Non-smokers (70)	25.223	6.9511	

Table (3-5): Platelet count in cigarette smokers according tothe number of cigarette/day.

Number of cigarette/day	Number of samples	Mean	Std. Deviation	P-Value
<20	54	238.3	45.233	0.768
≥20	46	235.3	55.771	

Table (3.6): Show platelet count according to the duration of smoking.

Duration of smoking(per year)	Number of samples	Mean	Std. Deviation	P value
≤ 10	79	238.30	51.798	0.584
>10	21	231.40	43.472	

Chapter four

4.1 Discussion:

This study was carried out to determine the effect of cigarette smoking on platelet count and indices in Sudanese male in Khartoum state. Blood samples were collected from 100 smokers and 70 samples from non-smokers as control group. The age of the participantsranged between 17- 55 years. The study conducted in the period from December 2013 to January2014.

The study revealed significant decrease in platelet count among smokers (p=0.000), this result agree with Butkiewicz who stated that platelet count was significantly lower in smokers. And disagree with results obtained by Gitte RN*et al* in India who used Beckman coulter automatic analyzer and Aghaji M. *et al* in Niger who reported that platelet count was significantly higher in smokers. Also disagree with the result obtained by Sirwansaksri J *et al*in Thai police and result of Parlak A and Kilci H who showed that platelet count were not significantly different between smokers and non-smokers.

The MPV was decreased in smokers but statistically not significant (p=0.219), this is similar to a study done by Arslan E., *et al*. But differ from the result obtained by Varol E In 2013 which showsthe values of MPV were significantly higher in smokers than non-smokers. Also disagree with result conducted by Parlak A and Kilci H who showed that MPV levels were higher in smokers than non-smokers but statistically not significant.

The result of PDW showed that there was no differences between smokers and non –smokers (p=0.178) , this is similar to the result of Parlak A and Kilci H who reported that there was no significant differences in PDW between both.

PLCR showed that there was no significant differences between smokers and non-smokers (p=0.269).

The effect of the smoking on platelet count is not related to the number of cigarette per day or to the duration of smoking(p= 0.768, p= 0.84 respectively) as shown in table (3-5 and 3-6).

4.2 Conclusion:

Our findings showed that there is statistical significant decrease in platelet count among cigarette smokers when compared with non-smokers. The effect of smoking on platelet count not associated with the number of cigarette or to the duration of smoking.

Platelet indices (MPV, PDW and PLCR) show no differences between smokers and non-smokers.

4.3 Recommendation:

1- Lower platelet count in smokers suggests that smoking is bad behavior so must be stopped.

2-Another study should be done to see the effect of smoking on platelet function and which of the component of cigarettehas the effect on platelet.

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Appendix (1)

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Faculty of Medical Laboratory Science

Department of Hematology

Questionnaire about:

Evaluation of the Effect of Cigarette Smoking on Platelet Count and Indices among Sudanese Smokers in Khartoum State.

No:…………………….. Date:…………………………

Name:………………….. Age: …………………………

Number of cigarettes /day: ………… Duration of smoking: ………….

Disease if present:

-Diabetes: ☐

-Hypertension: ☐

Result:

Platelet count: …………. /µL PDW:………………….fL

MPV: …………………..fL P-LCR: ………………. .%

Appendix (2)

KX-21N