Republic of Sudan
Ministry of Higher Education and scientific Research
Shendi University

Faculty of Graduate Studies and Scientific Research

Determination of Platelet Count and Mean Platelet Volume among Hypertensive Patients Living in Khartoum State

A thesis submitted for partial fulfillment of the requirement to award Master degree in Medical Laboratory sciences majoring Hematology

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1. Name: ……

2. Age: ……...

3.Gender :

Male ☐ **Female** ☐

4. Tel .No: …….

5. Duration of the disease :……..

Investigations performed :

i) **Platelet count :……...**

ii) **MPV :…….**

بسم الله الرحمن الرحيم

۞ وَقَضَىٰ رَبُّكَ أَلَّا تَعْبُدُوا إِلَّا إِيَّاهُ وَبِالْوَالِدَيْنِ إِحْسَانًا ۚ إِمَّا يَبْلُغَنَّ عِندَكَ الْكِبَرَ أَحَدُهُمَا أَوْ كِلَاهُمَا فَلَا تَقُل لَّهُمَا أُفٍّ وَلَا تَنْهَرْهُمَا وَقُل لَّهُمَا قَوْلًا كَرِيمًا (23) وَاخْفِضْ لَهُمَا جَنَاحَ الذُّلِّ مِنَ الرَّحْمَةِ وَقُل رَّبِّ ارْحَمْهُمَا كَمَا رَبَّيَانِي صَغِيرًا (24)

سورة الاسراء اية (23.24)

Dedication

To my beloved mother and father

To my sister and brothers

To my friends and everyone

who supported me.

Acknowledgment

All Thanks to Allah my God for he is always at my side , caring and supporting me through all stages of my life andto my dearparents encouraging me in all life occasions .

I am grateful to my supervisor Dr. Mouna Adel samaan from whomI learned a lot .she is a wonderful doctor and words can not express my gratitude to her.

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List of abbreviation

ADP	Adenine Diphosphate.
ATP	Adenine Triphosphate
BP	Blood Pressure.
CAMP	Cyclic Adenosine Monophosphate.
CBC	Complete Blood Count.
cDNA	ComplementaryDeoxyribo Nucleic Acid.
CHF	Congestive Heart Failure.
CVD	Cardiovascular Disease.
DBP	Diastolic Blood Pressure.
DIC	Disseminated Intravascular coagulation.
DNA	Deoxyribo Nucleic Acid.
EDTA	Ethylene Diamine Tetra Acetic acid.
FL	Femto- Liter.
GP	Glycoprotein.
HF	Heart Failure.
HLA	Human Leukocyte Antigen.
HPA	Human Platelet Antigen.
HT	Hypertension.
5-HT	5-hydroxytryptamine.

HTN	Hypertension.
LVH	Left Ventricular Hypertrophy.
MI	Myocardial Infarction.
MPC	Mean Platelet Component.
MPV	Mean Platelet Volume
PDGF	Platelet-Derived Growth Factor.
PF4	Platelet Factor 4.
PI 3 kinase	Phosphatidyl Inositol 3-kinase.
SBP	Systolic Blood Pressure.
SPSS	Statistical Package for Social Science.
TAT	Thrombin-Antithrombin Complex.
TPO	Thrombopoietin.
TSH	Thyroid Stimulating Hormone.
TXA2	Thromboxane A2.
vWF	von Willebrand Factor
US	United State.

ملخص البحث

أجريت هذه الدراسة على مرضى ضغط الدم من ولاية الخرطوم خلال الفترة من فبرايرإلى يوليو2018
أدرج في الدراسة 50 من المرضى بالإضافة إلى مجموعة 50 من لأفراد الذين كانواعلى ما يبدو يتمتعون بصحة جيدة ، والذين تم اختيارهم متنساقين في العمروالجنس. .هدفت هذه الدراسة .
الى الكشف عن التباين في عدد وحجم الصفائح الدموية في ارتفاع ضغط الدم وتحديد أهمية هذا التباين عند النظرفي متغيرات الدراسة المذكورة أعلاه.
تم ملء استبيان منظم لكل مريض ، وتم جمع عينة دم من الوريد واضافتها الى المضا د للتخثروالتي تم قياس عدد وحجم الصفائح الدمويه بها بعد ذلك.
وقد وجد أن متوسط عدد الصفائح الدموية في مجتمع الدراسة 284 ± 128.88 /مل 3 ومتوسط حجم .
الصفائح الدموية 10.34 ±1.47 فيمتو ليتر.
لم يتم الكشف عن أي فروق ذات دلالة إحصائية في متوسط عددالصفائح بين مجموعة الدراسة ومجموعة التحكم (p-value 0.634) .
بينما كان التغيرفي حجم الصفائح يعني أهمية إحصائية (p-value 0.027).
لم يتم الكشف عن اي اثار ذات دلاله احصائيه لمختلف فترات العمر والجنس ومدة المرض على متوسط عدد الصفائح الدموية وحجم الصفائح الدموية0

Abstract

This study was carried out on hypertensive patient recruited from Khartoum state during the period from February to July 2018.

Fifty hypertensive patients at different age intervals both males and females were included in this study . In addition to a control group of 50 apparently healthy individuals matching for age and sex were also tested.

The aim of this study was to detect the variation in platelets count and mean platelets volume in hypertensive patiant and to determine the significance of this variation when considering the above mentioned study variables .A well structured questionnaire was filled for each patient and EDTA anticoagulated veinous blood samples were collected on which platelet count and MPV were then measured .

It had been found that the mean platelets count in the study population was 284 ± 128.88/cmm and the mean platelets volume 10.34±1.47 fl . No statistically significant variation was detected in the mean platelets count between the study population and control group (P-value 0.634) , while as the variation in the mean platelets volume was statistically significant (P-value 0.027).

No statistically significant effects for different age intervals , gender and duration of the disease on the mean platelets count and mean platelets volume were detected .

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Introduction

1. Hypertension

Hypertension (HTN or HT) also known as high blood pressure (HBP), is a long term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure usually does not cause symptoms. Long term high blood pressure, however is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss and chronic kidney disease. Blood pressure is expressed by two measurements, the systolic and diastolic pressures, which are the maximum and minimum pressures, respectively. Normal blood pressure at rest is within the range of 100–140 millimeters mercury (mmHg) systolic and 60–90 mmHg diastolic. High blood pressure is present if the resting blood pressure is persistently at or above 140/90 mmHg for most adults. Different numbers apply to children. Ambulatory blood pressure monitoring over a 24-hour period appears more accurate than office best blood pressure measurement [1] .

This view has led the American Heart Association (AHA), to define the following ranges of blood pressure (in mmHg) [2] :

-Normal blood pressure is below 120 systolic and below 80 diastolic.

-Pre-hypertension is 120-139 systolic or 80-89 diastolic

-Stage 1 high blood pressure (hypertension) is 140-159 systolic or 90-99 diastolic.

-Stage 2 high blood pressure (hypertension) is 160 or higher systolic or 100 or higher diastolic.

-Hypertensive crisis (a medical emergency) is when blood pressure is above 180 systolic or above 110 diastolic [1,2] .

The disease burden of high blood pressure is a growing problem worldwide, in part because of a rapidly aging population. Other key contributors include lifestyle

factors, such as physical inactivity , a salt-rich diet associated with processed and fatty foods and Alcohol or tobacco use.

1.1.Risk factors of hypertension

Certain diseases and medications (as described below) can cause high blood pressure and there are a number of general risk factors for hypertension, including :

-Obesity : is a risk factor for high blood pressure and other cardiovascular conditions.

-Age: Prevalence of hypertension is higher in people over 60 years of age

-Race : African-American adults are at higher risk than white or Hispanic American adults.

-Sex : males and females have different risk profiles. While life time risk is the same for everybody, men are more prone to hypertension at a younger age and women have a higher rate of hypertension at older ages.

-Life style : greater intake of dietary salt, excessive alcohol, low dietary potassium and physical inactivity all contribute to an increased risk of hypertension [3] .

1.2. Causes and types of hypertension

1.2.1.Primary hypertension

Primary hypertension results from a complex interaction of genes and environmental factors . Numerous common genetic variants with small effects on blood pressure have been identified as well as some rare genetic variants with large effects on blood pressure [4] .GWAS have identified 35 genetic loci related to blood pressure; 12 of these genetic loci influencing blood pressure were newly found. Sentinel SNP for each new genetic loci identified has shown an association with DNA methylation at multiple nearby Cpg sites. These sentinel SNP are located within genes related to vascular smooth muscle and renal function. DNA methylation might affect in some way linking common genetic variation to multiple phenotypes even though mechanisms underlying these associations are not

understood. Single variant test performed in this study for the 35 sentinel SNP (known and new) showed that genetic variants singly or in aggregate contribute to risk of clinical phenotypes related to high blood pressure [4,5].

1.2.2.Secondary hypertension

Secondary hypertension results from an identifiable cause. Kidney disease is the most common secondary cause of hypertension. Hypertension can also be caused by endocrine conditions, such as Cushing's syndrome, hyperthyroidism, hypothyroidism, cromegaly, Conn's syndrome or hyper-aldosteronism, hyperparathyroidism and pheo-chromocytoma. Other causes of secondary hypertension include obesity, sleep apnea, pregnancy, coarctation of the aorta, excessive eating of liquorice, excessive drinking of alcohol and certain pre-scriptive medicines, herbal remedies and illegal drugs [6].

Arsenic exposure through drinking water has been shown to correlate with elevated blood pressure [6,7].

1.3.Signs and symptoms of hypertension

Hypertension is rarely accompanied by symptoms and its identification is usually through screening, or when seeking healthcare for an unrelated problem. Some with high blood pressure report headaches (particularly at the back of the head and in the morning), as well as lightheadedness, vertigo, tinnitus (buzzing or hissing in the ears), altered vision or fainting episodes [8].

These symptoms, however, might be related to associated anxiety rather than the high blood pressure itself [7,9] . On physical examination, hypertension may be associated with the presence of changes in the optic fundus seen by ophthalmoscopy. The severity of the changes typical of hypertensive retinopathy is graded from I- 6 frequently causes weight loss with increased appetite, fast heart rate, bulging eyes, and tremor. Renal artery stenosis (RAS) may be associated with a localized

abdominal bruit to the left or right of the midline (unilateral RAS), or in both locations (bilateral RAS). Coarctation of the aorta frequently causes a decreased blood pressure in the lower extremities relative to the arms, or delayed or absent femoral arterial pulses. Pheo -chromocytoma may cause abrupt ("paroxysmal") episodes of hypertension accompanied by headache, palpitations, pale appearance, and excessive sweating [8].

1.4. Complications of hypertension

The excessive pressure on the artery walls caused by high blood pressure
can damage blood vessels, as well as other organs in the body. The higher the blood pressure and the longer it goes uncontrolled, the greater the damage. Uncontrolled high blood pressure can lead to:

1.4.1.Complication effects on heart

Hypertensive heart disease is the result of structural and functional adaptations leading to left ventricular hypertrophy, diastolic dysfunction . Abnormalities of blood flow due to atherosclerotic coronary artery disease micro vascular disease and cardiac arrhythmias. Individuals with left ventricular hypertrophy are at increased risk for stroke, CHF and sudden death [11].

Control of hypertension can regress or reverse left ventricular hypertrophy and reduce the risk of cardiovascular disease . left ventricular hypertrophy are seen in 25% of the hypertensive patients and can be easily diagnosed by using echocardiography. Underlying mechanisms of hypertensive left ventricular hypertrophy are of 2 types: mechanical, mainly leading to myocyte hypertrophy; neuro-hormonal, mainly resulting in a fibroblastic proliferation [11,12]. Abnormalities of diastolic function, ranging from asymptomatic heart disease [13]. overt heart failure, are common in hypertensive patients. Patients with diastolic heart failure have apreserved ejection fraction, which is a measure of systolic function [14] .

Diastolic dysfunction is an early consequence of hypertension-related heart disease and is exacerbated by left ventricular hypertrophy and ischemia [11].

1.4.2.Complication effects in the brain

Hypertension is an important risk factor for brain infarction and hemorrhage [15]. Approximately 85% of strokes are due to infarction and the remainder are due to hemorrhage, either intra-cerebral hemorrhage or subarachnoid hemorrhage. The incidence of stroke rises progressively with increasing blood pressure levels, particularly systolic blood pressure in individuals >65 years. Treatment of hypertension convincingly decreases the incidence of both ischemic and hemorrhagic strokes [16] .

Hypertension is also associated with impaired cognition in an aging population ([17]. Hypertension-related cognitive impairment and dementia may be a consequence of a single infarct due to occlusion of a "strategic" larger vessel or multiple lacunar infarcts due to occlusive small vessel disease resulting in sub-cortical white matter ischemia [16,18] .

Several clinical trials suggest that anti hypertensive therapy has a beneficial effect on cognitive function, although this remains an active area of investigation .

Cerebral blood flow remains unchanged over a wide range of arterial pressures (mean arterial pressure of 50).

Diabetes has several complications of which one is hypertension or high blood pressure. Data indicate that at least 60-80 percent of individuals whom develop diabetes will eventually develop high blood pressure.

1.4.3.Complications affects on the kidneys

Hypertension is a risk factor for renal injury and end-stage renal disease (ESRD)[19]. Renal risk appears to be more closely related to systolic than to diastolic blood

pressure and black men are at greater risk than white men for developing ESRD at every level of blood pressure.

The atherosclerotic, hypertension-related vascular lesions in the kidney primarily affect the pre -glomerular arterioles [20] resulting in ischemic changes in the glomeruli and post glomerular structures. Glomerular injury may also be a consequence of direct damage to the glomerular capillaries due to glomerular hyper-perfusion. Glomerular pathology progresses to glomerulo-sclerosis, and eventually the renal tubules may also become ischemic and gradually atrophic. The

renal lesion associated with malignant hypertension consists of fibrinoid necrosis of the afferent arterioles [21] sometimes extending into the glomerulus, and may result in focal necrosis of the glomerular tuft. Clinically, macro-albuminuria (a random urine albumin/ creatinine ratio > 300 mg/g) or micro-albuminuria (a random urine albumin/creatinine ratio 30.

1.4.4.Hypertensive crisis

Severely elevated blood pressure (equal to or greater than a systolic 180

or diastolic of 110) is referred to as a hypertensive crisis. Hypertensive crisis is categorized as either hypertensive urgency or hypertensive emergency, according to the absence or presence of end organ damage respectively. In hypertensive urgency, there is no evidence of end organ damage resulting from the elevated blood pressure. In these cases, oral medications are used to lower the BP gradually over 24 to 48 hours. In hypertensive emergency, there is evidence of direct damage to one or more organs [22].

The most affected organs include the brain, kidney, heart and lungs producing symptoms which may include confusion, drowsiness, chest pain and breathlessness. In hypertensive emergency, the blood pressure must be reduced more rapidly to stop ongoing organ damage however, there is a lack of randomized controlled trial evidence for this approach[21,23].

1.4.5.Complications in pregnancy

Pre-eclampsia is a serious condition of the second half of pregnancy and following delivery characterized by increased blood pressure and the presence of protein in the urine . It occurs in about5% of pregnancies and is responsible for approximately 16% of all maternal deaths globally. Pre-eclampsia also doubles the risk of death of the baby around the time of birth. Usually there are no symptoms in pre-eclampsia and it is detected by routine screening. When symptoms of pre-eclampsia occur the most common are headache, visual disturbance (often "flashing lights"), vomiting, pain over the stomach, and swelling. Pre-eclampsia can occasionally progress to a life-threatening condition called eclampsia, which is a hypertensive emergency and has several serious complications including vision loss, brain swelling, seizures, kidney failure, pulmonary edema, and disseminated intravascular coagulation (a blood clotting disorder. In contrast, gestational hypertension is defined as new-onset hypertension during pregnancy without protein in the urine [24].

1.4.6. Strokes in hypertension

A stroke occurs when poor blood flow to the brain results in cell death. There are two main types of stroke: ischemic, due to lack of blood flow and hemorrhagic due to bleeding. Stroke result in part of the brain not functioning properly. The main risk factor for stroke is high blood pressure. Other risk factors include tobacco smoking, obesity, high blood cholesterol, diabetes mellitus, previous TIA, and atrial fibrillation [25] .

1.4.6.1.Ischemic stroke

Is typically caused by blockage of a blood vessel, though there are also less common causes . and may caused by interruption of the blood supply to the brain [25,26].These blockages are often caused by blood clots, which can form either in the arteries

connecting to the brain, or in other blood vessels before being swept through the bloodstream and into narrower arteries within the brain. Clots can be caused by fatty deposits within the arteries called plaque. In an ischemic stroke, blood supply to part of the brain is decreased, leading to dysfunction of the brain tissue in that area. There are three reasons why this might happen:

1-Thrombosis (obstruction of a blood vessel by a blood clot forming locally).

2- Embolism (obstruction due to an embolus from else where in the body, see below)

.

3-Systemic hypo perfusion (general decrease in blood supply, e.g., in shock) .

1.4.6.2.Hemorrhagic stroke

Is caused by either bleeding directly into the brain or into the space between the brain's membranes. Bleeding may occur due to a ruptured brain aneurysm [27].The leaked blood puts pressure on brain cells and damages them. Blood vessels can burst or spill blood in the middle of the brain or near the surface of the brain, sending blood into the space between the brain and the skull. The ruptures can be caused by conditions such as hypertension, trauma, blood-thinning medications and aneurysms (weaknesses in blood vesselwalls).

There are two main types of hemorrhagic stroke:

Cerebral hemorrhage (also known as intra-cerebral hemorrhage), which is basically bleeding within the brain itself (when an artery in the brain bursts, flooding the surrounding tissue with blood), due to either intra parenchymal hemorrhage (bleeding within the brain tissue) or intra ventricular hemorrhage (bleeding within the brain's ventricular system) [28] .

Subarachnoid hemorrhage, which is basically bleeding that occurs outside of the brain tissue but still within the skull, and precisely between the arachnoid mater and pia mater (the delicate innermost layer of the three layers of the meninges that surround the brain).

Intra-cerebral hemorrhage is the most common type of hemorrhagic stroke and occurs when brain tissue is flooded with blood after an artery in the brain bursts. Subarachnoid hemorrhage is the second type of hemorrhagic stroke and is less common. In this type of stroke, bleeding occurs in the subarachnoid space - the area between the brain and the thin tissues that cover it. Hemorrhagic strokes may occur on the background of alterations to the blood vessels in the brain, such as cerebral amyloid angiopathy, cerebral arterio venousmal formation and an intracranial aneurysm, which can cause intra-parenchymal or subarachnoid hemorrhage.

1.4.6.3.Signs and symptoms of stroke occurrence

Stroke symptoms typically start suddenly, over seconds to minutes and in most cases do not progress further. The symptoms depend on the area of the brain affected. The more extensive the area of the brain affected, the more functions that are likely to be lost. Some forms of stroke can cause additional symptoms. For example, in intracranial hemorrhage, the affected area may compress other structures. Most forms of stroke are not associated with a headache, apart from Sub-arachnoid hemorrhage and cerebral venous thrombosis and occasionally intra-cerebral hemorrhage.

The main symptoms of stroke are as follows:

-Confusion, including trouble with speaking and understanding .

-Headache, possibly with altered consciousness or vomiting ,

-numbness of the face, arm or leg, particularly on one side of the body , trouble with seeing in one or both eyes , trouble with walking including dizziness and lack of co-ordination.

Strokes can lead to long-term problems. Depending on how quickly it is diagnosed and treated, the patient can experience temporary or permanent disabilities in the aftermath of a stroke. In addition to the persistence of the problems listed above,

patients may also experience the following: -Bladder or bowel control problems , Pain in the hands and feet that gets worse with movement and temperature.

A stroke occurs when part of the brain is deprived of oxygen and nutrients, causing brain cells to die. Uncontrolled high blood pressure can lead to stroke by damaging and weakening the brain's blood vessels, causing them to narrow, rupture or leak. High blood pressure can also cause blood clots to form in the arteries leading to blocking blood flow and potentially causing a stroke. If the inner walls of a blood vessel (endothelium) are damaged, it makes it easier for blood clots to form. Damage to the endothelium exposes the underlying collagen in the blood vessel and this can promote clot formation by platelets [26-28] .

1.5.Platelets

Platelets also called thrombocytes (thrombo cyte, "blood clot cell"), are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clotting blood vessel injuries. Platelets have no cell nucleus: they are fragments of cytoplasm that are derived from the megakaryocytic of the bone marrow, and then enter the circulation.

1.5.1.Platelets Structure

Platelets are extremely small and discoid, 3.0 x 0.5 μm in diameter, with a mean volume 7-11 fl. The glycoprotein of the surface coat are particularly important in the platelet reactions of adhesion and aggregation which are the initial events leading to platelet plug formation during hemostasis. Adhesion to collagen is facilitated by glycoprotein Ia (GPIa). Glycoprotein Ib (defective in Bernard- Soulier syndrome) and IIb/IIIa (defective in thrombasthenia) are important in the attachment of platelets to von Willebrand factor (vWF) and hence to vascular sub endothelium where metabolic interactions occur. The binding site for IIb /IIIa is also the receptor for fibrinogen which is important in platelet-platelet aggregation. The plasma membrane

invaginates into the platelet interior to form an open membrane (canalicular) system which provides a large reactive surface to which the plasma coagulation proteins may be selectively absorbed. The membrane phospholipids (previously known as platelet factor 3) are of particular importance in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa).The platelet contains three types of storage granules: dense, α and lysosomes. The more frequent specific α granules contain a heparin antagonist (PF4), platelet-derived growth factor (PDGF), β-thromboglobulin, fibrinogen, vWF and other clotting factors. Dense granules are less common and contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), 5-hydroxytryptamine (5-HT) and calcium. Lysosomes contain hydrolytic enzymes and peroxisomes contain catalase. During the release reaction described below, the contents of the granules are discharged into the open canalicular system. Platelets are also rich in signaling and cytoskeletal proteins which support the rapid switch from quiescent to activation that follows vessel damage [29] .

1.5.2. Role of Platelets in Hemostasis

The main function of platelets is the formation of mechanical plugs during the normal hemostatic response to vascular injury. In the absence of platelets spontaneous leakage of blood though small vessels may occur. The immobilization of platelets at the sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet-platelet (aggregation) interactions. The blood flow conditions determine the specific receptor ligand interactions [29,30].

1.5.2.1.Platelet adhesion

The initiating event following vascular damage is platelet adhesion to exposed sub endothelial matrix proteins. The platelet glycoprotein (GP) receptors which mediate adhesion are dependent on the rate of shear. Under the intermediate to high shear

conditions found in arterioles, this event is strictly dependent on von Willebrand factor (vWF) and its receptor, the GPIb–IX–V complex. However, at the lower rates of shear found in the venous circulation and in the static conditions frequently used for experimental purposes, adhesion can occur directly to other sub endothelial matrix proteins such as collagen and fibrinogen, although vWF also supports this event in these vessels. In both cases, adhesion is strengthened considerably through activation of platelet surface integrins, which leads to an increase in affinity for their adhesive ligands. Adhesion applies also to recruitment of circulating platelets into the thrombus. vWF, exposed on the surface of the growing thrombus, also plays a fundamental role in this process, most notably at the high rates of shear that exist within arterioles and in diseased vessels. The platelet-bound vWF that supports these events is derived from plasma and via secretion from platelet α-granules. Adhesion to the growing thrombus is supported by binding of fibrinogen to the integrin αIIbβ3, a process that is more correctly termed aggregation [30] .

1.5.2.2.Platelet aggregation

Aggregation is used to describe cross-linking of platelets through binding of fibrinogen, or other bivalent or multivalent ligands such as vWF to the integrin αIIbβ3 on adjacent cells. In resting platelets, the integrin αIIbβ3 exists in a lowaffinity conformation that is unable to bind to vWF or fibrinogen at the concentrations found within plasma (although it is able to bind to immobilized forms of these two ligands under static or low shear conditions). Upon platelet activation, so-termed ‘inside-out’ signals from other receptors cause αIIbβ3 to undergo a conformational change that increases its affinity for fibrinogen, vWF and other RGD (arginine–glycine–aspartate)-containing ligands, including fibronectin and CD40 ligand (CD40L). Binding of fibrinogen and other ligands to αIIbβ3 promotes ‘outside-in’ signals that rein force platelet activation. The integrin can be activated through elevation of Ca2+ and by activation of protein kinase C, rapIb and

phosphatidylinositol 3-kinase (PI3 kinase) [30] .

1.5.2.3.Platelet release reaction and amplification

Primary activation by various agonists induces intracellular signaling, leading to the release of α and δ- granules. α -Granule contents play an important role in platelet aggregate formation and stabilization and, in addition, the ADP released from dense granules plays a major positive feedback role in promoting platelet activation. TXA2 is the second of the two major platelet positive feedback loops important in secondary amplification of platelet activation to firm a stable platelet aggregate. It is formed de novo upon activation of cytosolic phospholipase A2 (PLA2) which is the rate limiting step. This liberates arachidonic acid from the membrane phospholipids, and is metabolized by cyclooxygenase to TXA2. Thromboxane A2 not only potentiates platelet aggregation, but also has powerful vasoconstrictive activity. The release reaction is inhibited by substances that increase the level of platelet cAMP. One such substance is the prostaglandin prostacyclin (PGI2) which is synthesized by vascular endothelial cells. It is a potent inhibitor of platelet aggregation and prevents their deposition on normal vascular endothelium [30,31] .

1.5.2.4.Clot Formation and Retraction:

The highly localized enhancement of ongoing platelet activation by ADP and TXA2 results in a platelet plug large enough to plug the area of endothelial injury. In this platelet plug the platelets are completely degranulated and adherent to each other. This is followed by clot retraction which is mediated by GPIIb/IIIa receptors which link the cytoplasmic actin filaments to the surface bound fibrin polymers [31].

1.5.3. Measurement of platelet

platelet concentration is measured either manually using a hemocytometer, or by placing blood in an automated platelet analyzer using electrical impedance, such as a Coulter counter. The normal range (99% of population analyzed) for platelets in

healthy Caucasians is 150,000 to 450,000 per cubic millimeter (a mm3 equals a microliter). Or 150–400 × 109 per liter. The normal range has been confirmed to be the same in the elderly [29,30] .

1.5.4. Platelet indices

Mean platelet volume (MPV) is part of the Complete Blood Count (CBC) tests and it identifies the average size of platelets found in the blood of an individual. The test is specifically used to show the relationship between the production of platelets in the bone marrow or incidence on the destruction of platelets. Other Platelet indices like platelet distribution width (PDW), Plateletcrit (PCT) and Platelet large cell ratio (P-LCR) [32-34] .

1.6.Platelets and Hypertension

The endothelium produces a large number of substances that affect blood flow and in turn are affected by changes in the blood and the pressure of blood flow . Despite many therapeutic advances that have lead to increasingly effective 15 antihypertensive drug treatments, the precise patho physiological mechanisms of hypertension and its complications are still poorly understood. In hypertension, the delicate balance between the vasodilators and the vasoconstrictors is upset, leading to changes that then take place in the vascular beds, setting up a vicious cycle that further maintains the high blood pressure. There is also increasing evidence that platelets and the endothelium, which both get activated in hypertension, have a crucial role in the increased thrombotic tendency seen in hypertension. Indeed, despite exposure of the blood vessels to high pressures, the main complications of hypertension (that is, myocardial infarction and stroke) are paradoxically thrombotic in nature rather than hemorrhagic—"the thrombotic paradox of hypertension" or "Birmingham paradox." Certainly, increasing clinical and laboratory evidence suggests that hypertension per se may confer a pro-thrombotic or hyper-coagulable

state, with abnormalities of coagulation, platelets, and the endothelium—in fulfillment of Virchow's triad for thrombogenesis [35,36] . The processes of thrombogenesis and atherogenesis are also intimately related. Many components of the coagulation and fibrinolytic pathways are primary and secondary predictors of cardiovascular events. The close association of these markers with cardiac outcomes and common cardiovascular risk factors raises the distinct possibility that such indices are not merely markers or consequences of thrombosis, but may significantly contribute to the pathogenesis of arterial thrombotic disease. It is reported that high grade inflammatory diseases present with low levels of MPV, but low-grade inflammatory conditions present with high levels of MPV [37-40].

Literature Review

Many studies had been conducted to detect the relation between the platelets count and mean platelet volume in hypertension .No significant changes had been reported

concerning platelet count in hypertensive patients . All available data document the significant increase in MPV indicating active platelets and therefore high risk of possible thrombosis [41-43].

In China , cross sectional study was performed among 80545 medical checkup participants >18 years in age without hypertension or diabetes between April 2009 till May 2010 .It has been found that there is significant association between MPV and pre-hypertension [42] .

In India , a study to assess the MPV in patients with pre-hypertension and hypertension was conducted . This study included newly diagnosed and untreated 87 pre hypertensive patients, 30 hypertensive patients and 35 normotensive control subjects matched for age, gender, and body mass index. The MPV values of patients with pre-hypertension and hypertension were significantly higher than those of the control group (8.4 +/- 0.8 and 8.8 +/- 0.7 versus 7.9 +/- 0.5 fl; $p < 0.05$ and $p < 0.001$ respectively). It was also higher in hyper-tensives than in pre-hypertensives (8.8 +/- 0.7 versus 8.4 +/- 0.8 fl; $p < 0.05$). However, it was found that the presence of the hypertension (beta = 0.28, P = 0.003) was only significant predictors of higher MPV in a multivariable model that adjusted for other variables[43].

In Sudan , a study conducted[44] , concluded that hypertensive patients have no significant different in the mean of platelet count, PDW, MPV and P-LCR when compared with controls with (P-value 0.1, 0.6, 0.5 and 0.8 respectively) this is result done by Salma M I ,Babikre AM,2016 [44] .

Rational

Hypertension is the most important preventable risk factor for premature death worldwide .The value of platelet count and indices in the differential diagnosis of many disorders continues to be investigated . Variation in Mean Platelet Volume

reportedly in hypertension and it's associated complications as well as in diabetes mellitus, hyper-lipidemia, and coronary artery disease are of increasing interest to get deep understanding of the etiology of associated thrombosis . There is also an inverse relationship between MPV and associated cardiovascular outcome .

Objectives

General Objective

To determine the variation of platelets count and mean platelets volume in

Hypertensive patients living Khartoum state during the period from February to July 2018.

Specific objective

-To detect the variation in platelet count and mean platelets volume due to hypertension.

-To correlate the detected variation with age, gender, associated complication and treatment.

3.1. Study type and design

This is a descriptive cross-sectional study.

3.2. Study population

Patient with hypertension were included in this study .

3.2.1.Inclusion criteria

- Patients diagnosed with hypertension .
- Both gender (male and female).

3.2.2.Exclusion criteria

Patients should not be suffering from any other disorder .

3.3. Sample size

50 hypertensive patients in addition to 50 apparently healthy individual matching for age and sex ere included a as control group.

3.4. Study area

Patients living in Khartoum state were recruited in this study.

3.5. Methods and tools of data collection

Data were collected from each patient using structured questionnaire (see the appendix) , and by observation of laboratory experiments.

3.5.1.Blood Sample collection

Five ml of veinous blood sample were collected from each patient using sterile disposable syringe and applying aseptic , standardized and non-traumatic vein puncture technique . The sample was then emptied into an EDTA anticoagulant container.Blood was mixed with the anticoagulant by gently inverting the container several times and labeled with patient's name and serial number.

3.5.2. Measurement of platelets' count and indices

Platelet count and indices were measured immediately by exposing the well mixed EDTA anticoagulant veinous blood sample to a well-controlled fully automated haemocyto-meter (Sysmex KX 21N series SN B 2010) which works with electrical impedance principle , a method for counting cells. Whole blood is passed between two electrodes through an aperture so narrow that only one cell can pass through at

a time. The impedance changes as a cell passes through. The change in impedance is proportional to cell volume, resulting in a cell count and measure of volume .

3.6.Data analysis

The Statistical analysis was performed using Statistical Package for Social Science (SPSS11.5). The results were presented as mean ± standard deviation. Mean evaluation was performed using student T- test to determine the effect of hypertension on platelet count and MPV. P. value was considered statistically significant at <0.05 . Data were presented in form of tables and figures .

3.7.Ethical consideration

This study was approved by the ethical committee of the College of Graduate Studies , Shendi University . Beside , each patient gave a consent for this study.

Result

Fifty hypertensive patients in addition to 50 control healthy individuals matching for age and sex were included in thes study . The distribution of their gender and age were demonstrated in figure (4.1)& (4.2) .

Data as collected using structured questionnaire , the duration of disease were shown in figure ((4.3) .

Platelet count and mean platelet volume were measured in all test and control samples , the obtained result were shown in table (4.1) . The P-value for the effects of gender , age variation and duration of the disease on the measured parameters were demonstrated in tables (4.2) up to (4.4) respectively .

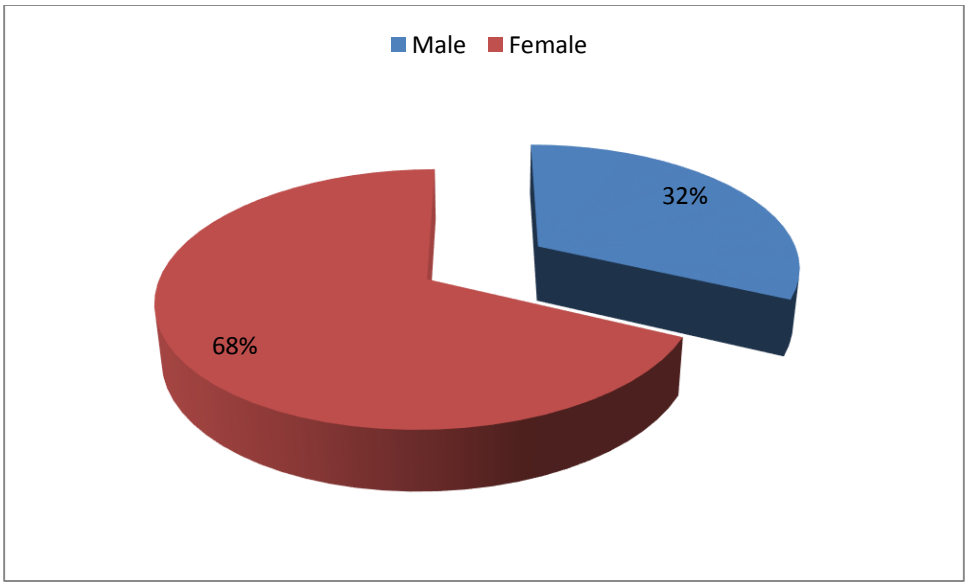
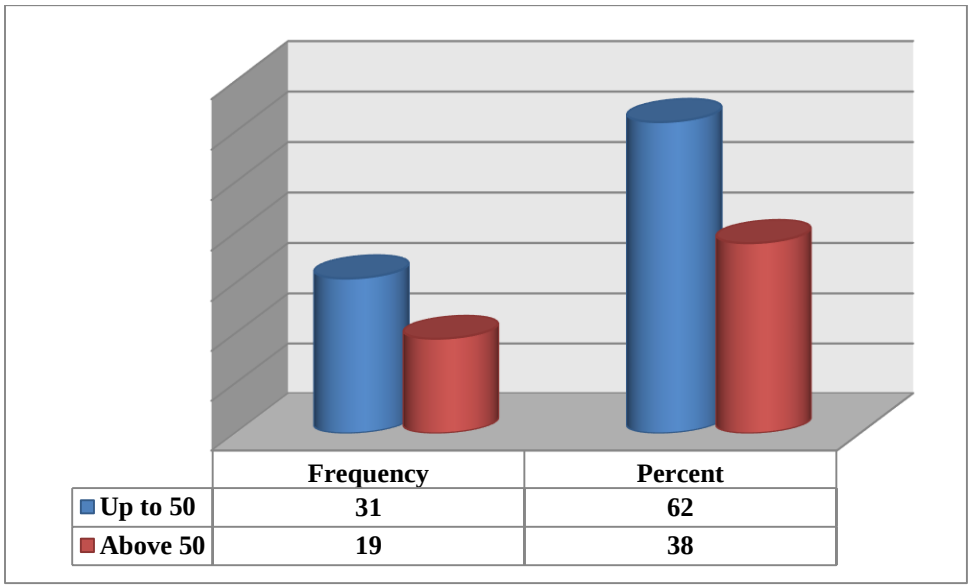


Figure (4.1) : The distribution of gender among the study population.



	Frequency	Percent
Up to 50	31	62
Above 50	19	38

Figure (4.2) : Distribution of hypertension patients according to age.

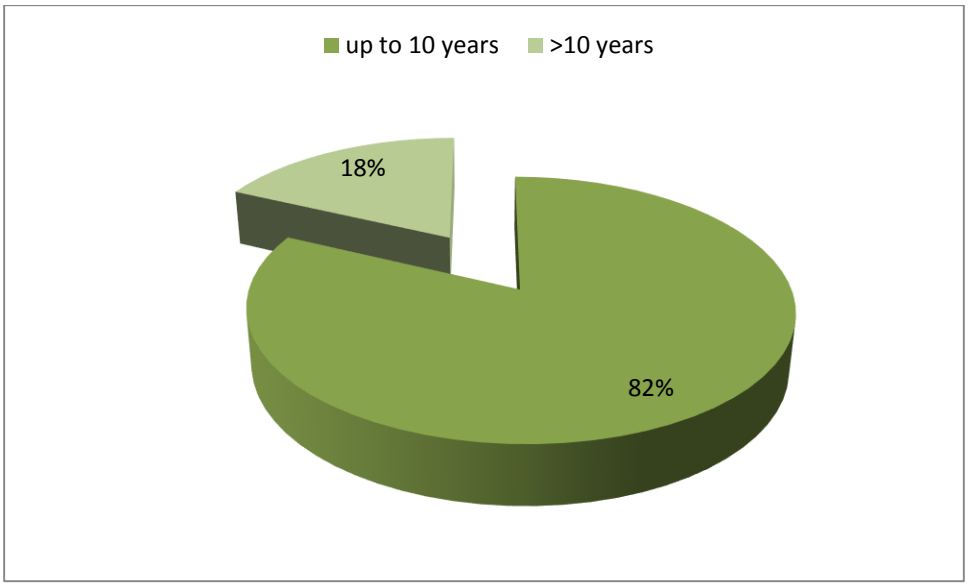


Figure (4.3) : Frequency of disease duration among the study population.

Table (4.1): The mean and P-value of platelets Count and MPV among patients and control.

Variables	Hypertension patient	Normal control	P. value
Platelet counts (/cmm) **Mean± Std**	284 ± 128.88	256± 65.2	0.634
MPV (FL) **Mean± Std**	10.34±1.47	13.45±16.5	0.027*

Table (4.2): According to the gender and platelets Count and MPV among patients.

Variables	Male	Female	P. value
Platelet counts (/cmm) **Mean± Std**	287.93± 128.3	282.26±189.2	0.500
MPV (FL) **Mean± Std**	10.2±1.3	9.82±1.23	0.176

Table (4.3) : The effect of age variation on platelets Count and MPV among patients.

Variables	Up to 50	More than 50	P. value
Platelet counts (/cmm) **Mean± Std**	279.25±125.8	291.94±185.6	0.306
MPV (FL) **Mean± Std**	9.48±0.98	9.04±1.04	0.121

Table (4.3): According to the age and platelets Count and MPV among patients.

Variables	Up to 10	More than 10	P. value
Platelet counts (/cmm) **Mean± Std**	266.16±123.15	356±134.08	0.369
MPV (FL) **Mean± Std**	10.46±1.03	9.82±.089	0.089

Discussion

A total of 50 hypertensive patients were reside from Khartoum state were included in thes study , in addition to a control group of apparently healthy individuals matching for both age and sex were also tested . Platelets count and mean platelets volume were assessed in all blood samples.

The mean platelet count in the study population and control were found to be 284 ± 128.88 /cmm and 256± 65.2/cmm respectively with a P-value of 0.634 . No statically significant variation was detected, indicating that hypertension has no effect on platelets count . This finding agree with the literature as no effect has been reported [41-43] .

As for the mean platelets volume, the measured results were 10.34±1.47 fl in the study population and 13.45±16.5fl in the control with a P-value of 0.027 indicating that hypertension increases the MPV. Again this matches with the literature reports as the increase in MPV can be considered as a useful indicator for stroke developing in hypertensive patients especially the uncontrolled type [42,43] . Certainly, increasing clinical and laboratory evidence suggests that hypertension per se may confer a pro-thrombotic or hyper-coagulable state with abnormalities of coagulation, platelets and the endothelium—in fulfillment of Virchow's triad for thrombogenesis . The close association of these markers with cardiac outcomes and common cardiovascular risk factors raises the distinct possibility that such indices are not merely markers or consequences of thrombosis, but may significantly contribute to the pathogenesis of arterial thrombotic disease. It is reported that high grade inflammatory diseases present with low levels of MPV, but low-grade inflammatory conditions present with high levels of MPV [40,41] .

No significant correlations or effects are detected for variation in the age intervals , gender and duration of the disease on platelet count or MPV . Nothing is reported in the literature concerning these variables .

6.1.Conclusion

-The mean platelets count in the study population was found to the normal range , and the mean platelets volume increase in hypertensive .

-No statistically significant variation was detected in the mean platelets count between the study population and control group.

-Statistically significant variation was detected in the mean platelets volume between the study population and control group .

-No statistically significant effects for different age intervals , gender and duration of the disease on the measured parameters .

6.1. Recommendation

1-Further study using large sample size can conducted in the future to :

A. confirm these findings in the platelets count and mean platelets volume .

B.determine the variation in MPV with associated clinical complication s.

2-Pre-hypertensive and hypertensive patients can be studied to get more definitive information on the effect of hypertension on platelets .

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