CHAPTER ONE

INTRODUCTION and LITERATURE REVIEW

1.1 Introduction

Cardiovascular disease (CVD) is a term for several linked pathologies, commonly defined as coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic and congenital heart diseases and venous thromboembolism. Globally CVD accounts for 31% of mortality, the majority of this in the form of CHD and cerebrovascular accident (WHO 2016). These diseases have sharply elevated during last two decades and continues to increasing; this may be associated with the growth in aging population and health risk behaviors (Jemal *et al.*, 2011). The role of inflammation in development and prognosis of cardiovascular diseases has been suggested by Folsom *et al*., 1999. Cardiovascular disease (CVD) is becoming the main cause of morbidity, mortality and disability in the world (Li-yuan *et al.*, 2015). Also it is becoming a large global burden the author mention that approximately one-third of all global deaths and 10% of total daily loss were attributed to CVD. For the past few decades, the majority of cardiovascular disease occurred in industrialized, higher-income countries. However, the absolute burden of cardiovascular disease has been greater in developing countries(Li-yuan *et al.*, 2015). CVD often accompanied with leukocytosis and it is thought to be associated with short term mortality and morbidity. The neutrophil count and NLR represent the balance between neutrophil and lymphocyte levels in the body and can be indicators of systemic inflammation. Some clinical trials have reported an association between increased absolute neutrophil count (ANC) in peripheral blood and short-term post-MI adverse

outcomes and worse angiographic findings also the value of monocyte count in predicting heart failure following MI. NLR may also reflect the myocardial remodeling responses after reperfusion injury(Li *et al.*,2016). In a study that investigated the relation between changes in CBC parameters and inflammation, a large increase in neutrophil counts and a pronounced decrease in monocyte and lymphocyte counts were observed(Jilma *et al.,* 1999); these alterations are correlate with mortality in acute compensated congestive heart failure, acute coronary syndrome and pulmonary embolism(Ceren *et al.,* 2016). On the other hand there is a positive correlation in nonspecific inflammatory conditions between the acute phase factors and proinflammatory proteins and a raised platelet count (Alexandrakis *et al.,* 2003). Moreover, the PLR has been presented as a potential indicator to detect excess thrombotic activity and inflammation in oncologic and cardiac disorders(Gürsoy *et al*.,2014; Kwon *et al*., 2012).

1.2. Literature Review

1.2.1. Definition of Cardiovascular diseases

Cardiovascular disease (CVD)defined as class of diseases that involve the heart or blood vessels (Shanthi *et al.,* 2011). Cardiovascular disease comprised of coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack) (Shanthi *et al* ., 2011). Other CVDs include stroke, heart failure, hypertensive heart disease ,rheumatic heart disease , cardiomyopathy , heart arrhythmia , congenital heart disease, valvular heart disease , carditis , aortic aneurysms ,peripheral artery disease , thromboembolic disease and venous thrombosis. The underlying process differed depending on the disease. Coronary artery disease, stroke, and peripheral artery disease contain atherosclerosis. This may be caused by high blood pressure, smoking, diabetes, lack of exercise, obesity,

high blood cholesterol, poor diet, and excessive alcohol consumption, among others. High blood pressure results in 13% of CVD deaths, while smoking results in 9%, diabetes 6%, lack of exercise 6% and obesity 5%. Rheumatic heart disease may follow untreated strep throat (Shanthi *et al.*, 2011). It is estimated that 90% of CVD is preventable. (McGill *et al.*, 2008)

1.2.2. Types of Cardiovascular diseases

There are several cardiovascular diseases which are classified according to WHO(WHO, 2016)

1.2.2.1. Coronary heart disease

Disease of the blood vessels supplying the heart muscle. Major risk factors: High blood pressure, high blood cholesterol, tobacco use, unhealthy diet, physical inactivity, diabetes, advancing age, inherited (genetic) disposition. Other risk factors Poverty, low educational status, poor mental health (depression), inflammation and blood clotting disorders.

1.2.2.2. Rheumatic heart disease

Damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria.

1.2.2.3. Congenital heart disease

Malformations of heart structures existing at birth may be caused by genetic factors or by adverse exposures during gestation. Examples are holes in the heart, abnormal valves, and abnormal heart chambers. Risk factors: Maternal alcohol use, medicines used by the expectant mother, maternal infections such as rubella, poor

maternal nutrition (low intake of folate), close blood relationship between parents (consanguinity).

1.2.2.4. Stroke

Strokes are caused by disruption of the blood supply to the brain. This may result from either blockage (ischaemic stroke) or rupture of a blood vessel (haemorrhagic stroke). Risk factors: High blood pressure, atrial fibrillation (a heart rhythm disorder), high blood cholesterol, tobacco usage, unhealthy diet, physical inactivity, diabetes, and advancing age.

1.2.2.5. Aortic aneurysm and dissection

Dilatation and rupture of the aorta. Risk factors: Advancing age, long-standing high blood pressure, Marfan syndrome, congenital heart disorders, syphilis, and other infectious and inflammatory.

1.2.2.6. Peripheral arterial disease

Disease of the arteries supplying the arms and legs. Risk factors: As for coronary heart disease.

1.2.2.7. Deep venous thrombosis (DVT) and pulmonary embolism

Blood clots in the leg veins, which can dislodge and move to the heart and lungs. Risk factors: Surgery, obesity, cancer, previous episode of DVT, recent childbirth, use of oral contraceptive and hormone replacement therapy, long periods of immobility, for example while travelling, high homocysteine levels in the blood.

1.2.2.8. Other cardiovascular diseases

These diseases include: Tumors of the heart; vascular tumors of the brain; disorders of heart muscle (cardiomyopathy); heart valve diseases; disorders of the lining of the heart. Other factors that can damage the heart and blood vessel system Inflammation, drugs, high blood pressure, unhealthy diet, trauma, toxins and alcohol.

1.2..3. Risk Factor of cardiovascular diseases

1.2.3.1. Genetics

Genetic factors affect the occurrence of cardiovascular disease in males who are less than 55 years-old and in women who are less than 65 years old. (McPhee *et al.*,2012). Cardiovascular disease in an individual's parents elevates their risk by 3 fold (Kathiresan *et al.*,2012). Multiple single nucleotide polymorphisms (SNP) have been found to be associated with cardiovascular disease in genetic association studies, but usually their individual influence is tiny, and genetic denoting to cardiovascular disease are poorly studied(Nikpay *et al.*, 2015; MacRae *et al.*, 2016).

1.2.3.2. Age

Age is the most crucial risk factor in occurring cardiovascular or heart diseases, with approximately a tripling of risk with each decade of life(Finegold *et al.*, 2012). Coronary fatty streaks can start to appear in adolescence (D'Adamo *et al.*, 2015). About 82 percent of people who die of coronary heart disease are 65 and older. Simultaneously, the risk of stroke duplicated every decade after age 55 years (Mackay *et al.*,2004). Multiple justifications are suggesting explaining why age

increases the risk of cardiovascular/heart diseases. One of them relates to serum cholesterol level (Jousilahti *et al.*,1999). In most populations, the serum total cholesterol level increases as age increases. In males, this elevated levels of around age 45 to 50 years. In females, the raising continues pointedly until age 60 to 65 years (Jousilahti *et al.*, 1999). Aging is also correlated with altering in the mechanical and structural features of the vascular wall, which leads to the loss of arterial elasticity and reduced arterial compliance and may subsequently lead to coronary artery disease (Jani *et al.*,2006). WHO,2016 estimated that over 75% of premature CVD is preventable and risk factor improvement can help decrease the rising of CVD burden on both individuals and healthcare providers. While age is a known risk factor for the development of CVD, the procedure of developing CVD in later years is not inevitable, thus risk reduction is crucial.

1.2.3.3. Gender

Women develop heart disease some directs the formation of testes rather than ovaries, years later than men. This raises the question of whether and the testes in turn produce testosterone and dihydrotes there is some aspect of 'femaleness' which reduces risk, or tosterone rather than estrone and estradiol as the primary whether there is some aspect of 'maleness' that raises risk. To date, most attention has been focused on the hypothesis drive down the HDL cholesterol levels, with consequent that endogenous estrogen is cardioprotective in women (Mendelsohn *et al.*, 1999). Rising rates of coronary heart disease (CHD) after the CHD may well be due the most basic genetic difference menopause, and after oophorectomy, are among the strands of evidence in humans that endogenous estrogen may chromosome. prevent CHD (Colditz *et al.*, 1987). Same studies reported that the prevalence of cardiovascular disease is greater in men than in women (Lemer

1986; Wingard *et al.*, 1983). The reason for this difference among genders is not fully understood. Sex hormones are likely to be involved in the relative protection from cardiovascular disease noted in women before menopause (lemer 1986). Accordingly, changes in hormones occurring during menopause are associated with an increased risk of cardiovascular disease and after menopause, the prevalence of cardiovascular disease progressively increases to the levels found in men(Witteman *et al.*, 1989).

1.2.4. Pathophysiology

Population-based studies reveal that atherosclerosis, the major origin of cardiovascular disease, start in childhood. The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study showed that intimal lesions appear in all the aortas and more than half of the right coronary arteries found in youths aged 7–9 years (Vanhecke *et al.*,2006). This is extremely crucial considering that 1 in 3 people die from complications related to atherosclerosis. Also stem the tide, education and awareness that cardiovascular disease considered as the greatest threat, and measures to inhibit or reverse this disease must be taken. Increased body index and diabetes mellitus are often linked to cardiovascular disease, (Highlander *et al.*, 2015) as are a history of chronic kidney disease and hypercholesterolaemia (Medicinewise 2011). In fact, cardiovascular disease is the most life-threatening of the diabetic complications and diabetics are two- to four fold more likely to die of cardiovascular-related causes than non diabetics (Norhammar *et al.*, 2004;Kvan *et al.*,2007).

1.2.5. Epidemiology

Cardiovascular diseases are the leading cause of death worldwide and in all regions with exception of Africa(Shanthi *et al.*,2011). In 2008, 30% of all global mortality was belonged to cardiovascular diseases. Death caused by cardiovascular diseases are also increased in low- and middle-income countries as over 80% of all global deaths caused by cardiovascular diseases occurred in those countries. It is also estimated that by 2030, over 23 million people will die from cardiovascular diseases each year. It is assessed that 60% of the world's cardiovascular disease burden will occur in the South Asian subcontinent in spite of only accounting for 20% of the world's population. This may be secondary to a combination of genetic and environmental factors. Heart disease is prevalent in Sudan, with at least 2.5% of the population affected, and it is one of the major causes of hospital mortality (Suliman *et al*,.2011).

1.2.6. Blood

Blood is specialized liquid connective tissue (Chauhan 2013), pumped by the heart through arteries and veins reaching all body's cells (Mehta and Hoff brand 2014).

1.2.6.1. Functions

Transport and distribute oxygen, nutrients, hormones and waste products, regulate pH, osmotic pressure and body temperature, control blood loss by assistance of platelets and coagulation factors and involves in body's immune response which mediated by leukocytes (Cheesbrough 2005).

1.2.6.2. Constituents of blood

It consists of cells (erythrocytes and leukocyte) and cell fragments(platelets), surrounded by liquid extracellular matrix called plasma(Chauhan 2013). Plasma forms about 55% of blood volume and contains water (95%) and many solutes, including proteins, mineral ions, organic molecules, hormones, enzymes, products of digestion and waste products for excretion. (Cheesbrough 2005).

1.2.6.3. Structure and Function of mature red blood cells (RBCs)

RBCs area discoid shape have specialized membrane flexibility which provide large surface areas for gas exchange, and allow repeated passes through narrow capillaries. RBCs lack nuclei and other organelles. These unique differences enabling maximal cytoplasmic occupation by hemoglobin(Palis 2014; Blann and Ahmed 2014).

1.2.6.4. Red blood cells count

Assessment of the RBC is to check for anemia and to evaluate normal erythropoiesis. The number of red blood cells is determined by age, sex, altitude, diet, drug use, tobacco/nicotine use and health and disease status (Lokwani 2013).

1.2.6.5. Hematocrit and red cell indices

The hematocrit is one of the most precise methods, used for red cell disorders differentiation and determination of the degree of anemia or polycythemia (Lokwani 2013). The red blood cell indices (MCV, MCH, and MCHC) provide information concerning the size and hemoglobin content of red blood cells (Ciesla 2012). RDW is quantitative measure of variation in red cells size. It has been proposed to be useful in early classification of anemia as it becomes abnormal

earlier in nutritional deficiency anemia than other red cell parameters (Greer *et al.*,2014).

1.2.6.6. Hemoglobin (Hb)

Hemoglobin molecule is composed of iron containing pigment called (heme) and protein (globin)(Ramadas 2012).It binds efficiently to oxygen molecules, and somewhat less efficiently to carbon dioxide molecules, thereby functioning in the transport of gases through the bloodstream.(Palis 2014).Hemoglobin is measured to detect anemia and its severity and to monitor an anemic patient's response to treatment.(Cheesbrough 2005).

1.2.6.7. Leukopoiesis

It is the process by which white blood cells are produced. It has three lines of cell development Lymphopoiesis(production of lymphocytes), Monopoiesis (production of monocytes) and granulopoiesis(production of granulocytes) (Blann and Ahmed, 2014).

1.2.6.8. Lymphopoiesis

The main sites of lymphopoies are the primary lymphoid tissues (thymus for T lymphocyte and bone marrow for B lymphocyte) .It may also occur peripherally in the lymph nodes, spleen and peyer patches in the intestine (secondary lymphoid tissues) (Ciulla and Lehman, 2010).Lymphocytic maturation is divided into three stages: the lymphoblast, prolymphocyte and lymphocyte (large and small) (Rozenberg 2011).

1.2.6.8.1. Structure and Function of mature lymphocytes

The nucleus is round, with coarse clumped chromatin. The cytoplasm is scanty, sky blue in color and a granular (Rozenberg 2011). B lymphocytes are responsible for antibodies production and T cells are in charge of cell mediated immunity and regulate the function of immune system cells (Hillman *et al.*, 2010).

1.2.6.9. Granulopoiesis

Granulocytes are formed in the bone marrow from a common precursor cell. In the granulopoietic series progenitor cells, myeloblasts, promyelocytes and myelocytes form a proliferative or mitotic pool of cells while the metamyelocytes, band and segmented granulocytes make up a post-mitotic maturation compartment (Hoff brand and Moss, 2016).

1.2.6.9.1. Structure and functions of mature granulocytes

1.2.6.9.1.1. Neutrophil

The nucleus is lobulated, having three or four lobes connected by thin strands of chromatin. The cytoplasm is pink and contains specific granules (Rozenberg 2011).It locates and eliminates pathogens and regulates immunity and inflammation. It also proposed to have a role in protection against intracellular pathogen (Mocsai 2013).

1.2.6.9.1.2. Eosinophil

Eosinophil is characterized by its two-lobed nucleus and red orange staining granules. It has significant proinflammatory and cytotoxic activity and plays a role

in the pathogenesis of various allergic and parasitic disorders(Howard and Hamilton, 2013).

1.2.6.9.1.3. Basophil

It has large dark purple granules which may conceal the nucleus. The granule contents include histamine and heparin (Mehta and Hoff brand, 2014). It plays a central role in immediate hypersensitivity reactions (Howard and Hamilton, 2013).

1.2.6.10. Monopoiesis

Monocytes are derived from progenitor cells in the bone marrow(Turgeon 2012). The maturation of monocytes is divided into three stages: the monoblast, promonocyte and mature monocyte (Rozenberg 2011).

1.2.6.11.WBC count and differential WBC count

White blood cell (WBC) count is used in investigation of infection, bone marrow disorders, immune system disorder and reaction to a drug(Lokwani 2013).Also used to monitor treatments which can cause leucopenia (Cheesbrough 2005). Differential white cell count is the relative percentage of each cell type (Greer 2013); thereby provides information the different white cells present in the circulating blood. Providing the total WBC count is known, the absolute number of each white cell type(number of each cell per liter of blood), can be calculated and an assessment made of whether the number of a particular cell type is increased or decreased(compared with the reference range) (Cheesbrough 2005).

1.2.6.12. Megakaryopoiesis

It is the process of development of megakaryocytes and platelets in the bone marrow (Kawthalkar 2013). Megakaryocytic maturation is divided into three stages those are megakaryoblast, promegakaryocyte and megakaryocyte. (Rozenberg 2011). Megakaryocyte development characterized by nucleus duplication (polyploidization), cytoplasmic maturation and expansion, (Deutsch and Tomer, 2006) and terminal thrombopoiesis with proplatelet formation and platelet release (Tozawa 2014).

1.2.6.12.1. Structure and function of platelet

Platelet is small a nucleated cell fragment has discoid shape maintained by a band of parallel microtubules. It has two tubular systems: the dense tubular system and the surface-opening canalicular system. Also contain two types of granule alpha and delta. Platelet play role in managing and regulating hemostasis (Ghoshal and Bhattacharyya., 2014).

1.2.6.12.2. Platelet count

A platelet count used to investigate abnormal skin and mucosal bleeding. Platelet counts are also performed when patients are being treated with cytotoxic drugs or other drugs which may cause thrombocytopenia (Cheesbrough 2005).

1.2.6.12.3. Platelet parameters

Platelet parameters include (MPV, PDW and P-LCR) which measure size of platelet and its variation. These parameters provide an indicator to abnormal bone marrow function and periphery destruction of platelet another parameter of platelet is plateletcrit which has no clinical value (Bain *et al.*, 2011).

1.2.6.13. Role of neutrophil to lymphocyte ratio

Systemic inflammation can be measured by using a variety of biochemical and hematological markers. Although novel disease specific biomarkers have been identified, most of which are time consuming and expensive. Observational studies have thoroughly investigated the role of C-reactive protein and total leukocyte count in different chronic conditions (Bovill *et al.*,1996; Flosom *et al.,* 2002). Recent evidence indicated that the ratio of sub types of blood cells have a significant prognostic value for cardiovascular disease. Elevated levels of neutrophil lymphocyte ratio (NLR) were also found associated with poor survival of patients undergoing coronary artery bypass graft (Gibson *et al.,* 2007).

By contrast, lymphocytes play vital roles in the remodeling of the myocardium following inflammation. For example, CD4+ T regulatory cells constitute a particularity-inflammatory immune regulatory lymphocyte subset which is generated in the thymus and highly enriched for T cells with auto antigen specificity (Stephenson *et al.*, 2017). T cells are essential for the recruitment of proangiogenic macrophages and collateral artery formation (Tang *et al.*, 2012). B cells are involved in monocyte recruitment through the CCL7 pathway (Stephenson *et al.*, 2017). The clearance of debris, activation of fibroblasts and collagen deposition for scar formation and neovascularisation (the proliferative phase) occur (Ruparelia *et al.*, 2017; Ong *et al.*, 2018) days after MI (Frangogiannis 2008, 2014; Prabhu *et al.,* 2016).

The release of inflammatory and anti-inflammatory mediators IL-10, TGF and proresolving mediators (Prabhu *et al.*, 2016; Zlatanova *et al.*,2016) from neutrophil or lymphocyte cells promotes neutrophil apoptosis and phagocytic up take by macrophages (Kolaczkowska *et al.*, 2013; Horckmans *et al.*, 2017) IL-10 secreted

by T lymphocytes inhibits the production of inflammatory cytokines, stabilizes the matrix and regulates ECM metabolism. Macrophages engulfing apoptotic neutrophils are a key activator of the anti-inflammatory response and potent inhibitor of pro-inflammatory cytokines. Neutrophils are seen as a marker of ongoing inflammation and lymphocytes as a marker of regulatory pathways. Neutrophil-to-lymphocyte ratio (NLR) (calculated via dividing neutrophil count by lymphocyte count) as an indication of systemic inflammation has been demonstrated to be associated with poor clinical outcomes in various cardiovascular diseases, including acute coronary syndrome. Recent accumulating evidence points that high NLR to be independently and strongly associated with increased risk of complications and mortality post-acute MI (Karakas *et al.*, 2016).

1.2.6.14. Role of platelets to lymphocyte ratio

The interaction of inflammation and thrombosis is the critical factor in the pathogenesis of ACS (Falk *et al.,* 2006). As a reflection of excess inflammatory status and thrombotic activity, elevated peripheral blood platelet count is regarded to be a valuable predictor of adverse cardiovascular outcomes. And multiple researches have demonstrated this speculation. On the other hand, decreased lymphocyte count is also related to worse cardiovascular prognosis, which may be partly explained by the role of lymphocyte in protection of plaque stability (Li *et al.*,2016). A novel marker, platelet to lymphocyte ratio (PLR), seemed to be a potential indicator in CVD prognosis. Recently, the prognostic importance of PLR has been investigated by several studies (Li *et al.*,2016).

PLR was positively associated with the Global Registry of Acute Coronary Events (GRACE) risk score and can improve its the predictive power for long-term cardiovascular events in patients with ACS (Zhou *et al.* 2016). Also, PLR was an

independent predictor of in-hospital cardiovascular mortality in patients with ST-elevated acute myocardial infarction (Temiz *et al.*, 2014). A significant association between high PLR levels and the adjusted risk of 6-month all-cause deaths in ST elevation myocardial infarction underwent primary coronary intervention, while with no difference in hospital mortality among patients with different PLR levels (Ugur,\ *et al.*, 2014). Although most studies demonstrated the positive association between PRL and CVD adverse outcomes, there are still some discrepancies.

1.2.7. Previous studies

Demirkol *et al*.(2012) reported significant increase in NLR ratio in patients with Cardiac Syndrome X(CSX) and CAD, compared to the control group. They added that patients with CAD and CSX had significantly higher C-IMT value compared to control participants. Significant positive correlation was found between C-IMT (intima-media thickness) value and plasma level of N/L ratio (Demirkol *et al*., 2012). Yue *et al.*(2015) found that the mean NLR was significantly higher in patients with diabetic retinopathy compared with patients without DR ($p < 0.01$) (Yue *et al.*, 2015). Chen Chen *et al.*(2018) investigate that NLR in peripheral blood is established to correlate with the morbidity and mortality of heart disease patients, NLR positively correlated with myocardial damage but negatively correlated with myocardial function (Chen Chen *et al.*, 2018). Imtiaz *et al.* (2012)determine the neutrophil lymphocyte ratio in prevalent chronic disease and they conducted to proportion of individuals with hypertension was higher in middle and highest tertile of NLR as compared to the lowest tertile (Imtiaz *et al.*, 2012).

Cardiac arrests were observed more frequently in male patients, while the number of non-cardiac arrests was greater in female patients. PLR and NLR were higher in non-cardiac arrests than in cardiac arrests.

1.3. Rationale

Cardiovascular diseases are most popular common chronic disease. The total white blood cell count (WBC) and its subtypes, neutrophil to lymphocyte (N/L) ratio, can be used as an indicator of systemic inflammation. The N/L ratio has been demonstrated to have the greatest predictive power of death, cardiac disease and other complications. Elevated N/L ratio is an independent predictor of long-term inflammation can be measured by using a variety of biochemical and hematological markers. Although many specific biomarkers have been identified, most of which are time consuming and expensive. Recent evidence indicated that the ratio of sub types of blood cells have a significant prognostic value for cardiovascular disease. This study will be done to evaluate this relationship

1.4. Objectives

1.4.1 General objective

To calculate neutrophil lymphocyte ratio (NLR) and platelet lymphocyte ratio(PLR) among Sudanese cardiovascular disease patients

1.4.2 Specific objectives

-To assess CBC parameters in patient with cardiovascular disease.

-To measure NLR in patient with cardiovascular disease.

-To measure PLR in patient with cardiovascular disease.

-To investigate the effect of age, gender, and type of CVD on NLR and PLR in CVD patients.

-To compare between NLR and PLR as inflammatory markers patient with cardiovascular diseases

CHAPTER TWO

MATERIALS AND METHODS

2.1. Study Design

This is cross-sectional study, designed to measure the NLR\PLR as a markers for systemic inflammation in CVD patients.

2.2. Study area and duration

This study was conducted in Khartoum state at Sudan Centre for Heart Care and Almoalim Medical City, during the period from August-December 2018.

2.3. Data collection

Samples were collected randomly, laboratory investigations were performed for CBC, also questioner was conducted in this study.

2.4. Study population

200 samples were partispate in this study, with different age and gender. 100 samples from patients diagnosed with cardiovascular diseases and other 100 samples from normal healthy individuals were used as control.

2.5. Criteria of Selection

2.5.1. Inclusion Criteria

Patients who have cardiovascular diseases regarding of age, gender and type of disease.

2.5.2. Exclusion Criteria

Patients who are diabetic, renal disease patients and diseases other than cardiovascular diseases.

2.6. Sample collection

3ml of venous blood sample was collected in ethylenediaminetetra-acetate (EDTA) evacuated tube for blood analysis.

2.7. Sample analysis

Complete blood counts, which included total WBC, neutrophils, and lymphocytes, were obtained at the time of admission. NLR was calculated as the ratio of neutrophil count to lymphocyte count, and PLR was calculated as the ratio of platelet count to lymphocyte count. The CBC (WBC, Neutrophils , Lymphocytes and platelet count) was measured by using automated hematological analyzer (sysmex XP-300).

2.8. Methods

2.8.1. Sysmex XP-300

This automated hematological analyzer measures the cell counts using direct current (DC) detection method and measures Hb concentration by Non-Cyanide method.

2.8.1.1. Direct Current detection method

Blood sample is aspirated, measured to predetermined volume, diluted at the specified ratio, and then fed into each transducer. The TD chamber has a minute

hole called the aperture. On both sides of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes as direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses.

2.8.1.2. Quality control

The reliability of this instrument and reagents is monitored by controls and calibrators using of control or blood or control materials the stability of the measured value is monitored over a certain period of time, and problems can be detected early or prevented.

2.9. Data analysis

The obtained data was encoded and entered in Microsoft Excel sheet and then analyzed by using Statistical Package for Social Science program v. 21.0 for windows (SPSS Inc, Chicago, IL, USA). The analyzed data presented in tables and figures designed by Microsoft Excel 2007. ANOVA test was used as significance test and to assess the factors associated with NLR and PLR. The P. value was considered as significant in the level of 0.05.

2.10. Ethical consideration

Consent of selected individuals to study was taken after being informed with all detailed objectives of the study.

CHAPTER THREE

RESULT

The study population consisted of 100 patients as with CVD as case group and 100 individuals without CVD as control group. The majority of the case group 52(52%) found in age group more than 60 years, and the majority of control group 45(45%) found in age group from 40-60 years (P= 0.000) (table 1)

In the gender of the study population, the majority of the case group 65 (65%) were males while near to one-half (51%) of control group were females (P= 0.016) (table 2)

Concerning the types of CVD among the case group, 70(70%) had STEMI, 15(15%) had non-STEMI and also 15(15%) had unstable angina (figure1)

Regarding to the NLR, the case group showed significantly higher value of NLR when compared to control group (5.6±3.3 vs. 1.7±0.8; P= 0.000) (figure 2)

Likewise, the case group showed significantly higher value of PLR when compared to control group (162.3±83.2 vs. 98.4±28.1; P= 0.000) (figure 3)

As illustrated in table 3, the age of the CVD patient showed insignificant association with both NLR (P= 0.729) and PLR (0.40)

The correlation between NLR and PLR with the gender of CVD patients showed that, NLR was significant higher in male (6.7±4.7) than female (3.9±2.4), the difference was statistically significant (P= 0.008). Also PLR was significant higher in male (182.5±62.2) than female (156.6±67.4), and the difference was statistically significant (P= 0.013) (table 4).

Concerning to the correlation between NLR and PLR with the type of CVD patients, the highest value of NLR was found in non-STEMI patients (6.8±5.9) and lowest value in the patients with unstable angina (2.9±1.8), the difference was statistically significant (P= 0.003). Similarly, the highest value of PLR was found in non-STEMI patients (219.0±126.1) and lowest value in the patients with unstable angina (147.3±68.3), the difference was statistically significant (P= 0.007) (table 5).

In the comparison of CBC findings among the study groups, the mean value of hemoglobin for case group was 10.6 g/dl ± 1.6 and 14.4 mg/dl ± 2.5 for control group, and the difference was statically significant (P= 0.000). The mean value of PCV for case group was 32.4% ± 5.3 and 40.3 % ± 1.8 for control group, and the difference was statically significant (P= 0.000). The mean value of WBCs count for case group was 8.9x103/µL ± 1.8 and 5.4x103/µL ± 1.0 for control group, and the difference was statically significant (P= 0.000). The mean value of platelets count for case group was 316x103/µL ± 92.2 and 286x103/µL ± 50.4 for control group, and the difference was statically significant (P= 0.000). The mean value of neutrophil count for case group was 6.8x103/µL ± 1.5 and 2.7x103/µL ± 0.9 for control group, and the difference was statically significant (P= 0.000). The mean value of lymphocyte count for case group was 1.8x103/µL ± 0.8 and 2.0x103/µL ± 0.5 for control group, and the difference was statically significant (P= 0.001) (table 6).

Table 1: The distribution of the age among the case and control groups

Age (Years)	**Group**		**P. value**
	Case (N= 100)	**Control (N= 100)**	
<40	15	40	0.000*
	15.0%	40.0%	
40-60	33	45	
	33.0%	45.0%	
>60	52	15	
	52.0%	15.0%	

Chi-Square test was used

*P. value is significant at level 0.05

Table 2: The distribution of the gender among the case and control groups

Gender	**Group**		**P. value**
	CVD (N= 100)	**Control (N= 100)**	
Male	65	49	0.016*
	65.0%	49.0%	
Female	35	51	
	35.0%	51.0%	

Chi-Square test was used

*P. value is significant at level 0.05

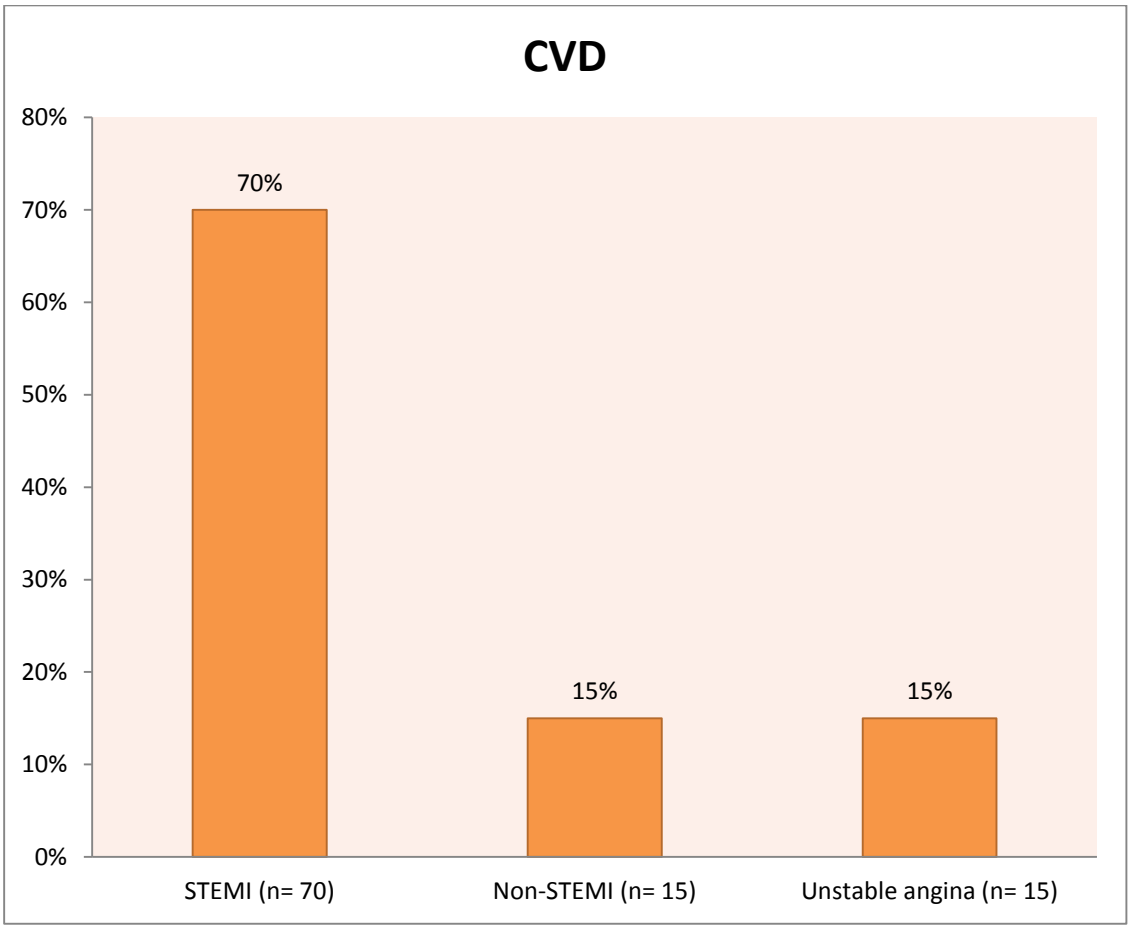

Figure1: the types of CVD among the case group (N= 100)

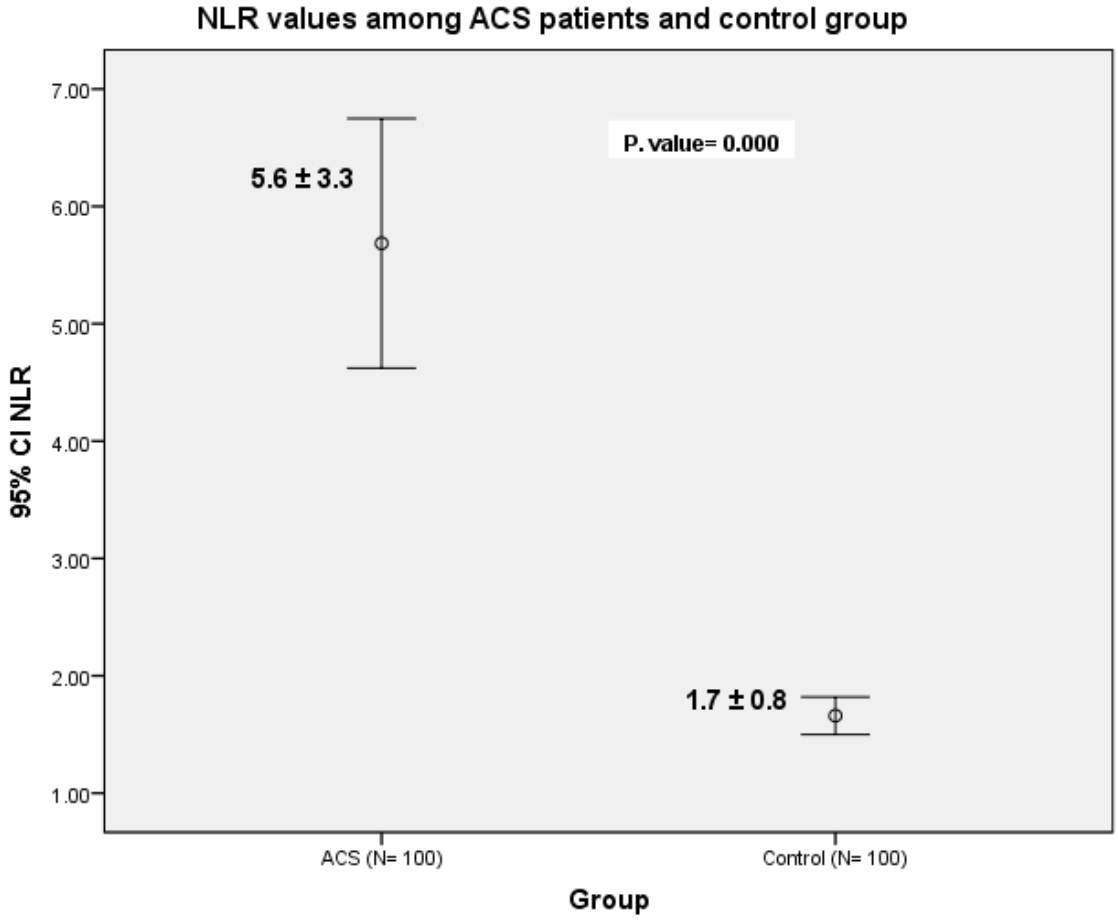

Figure 2: The comparison of NLR values among the case and control groups

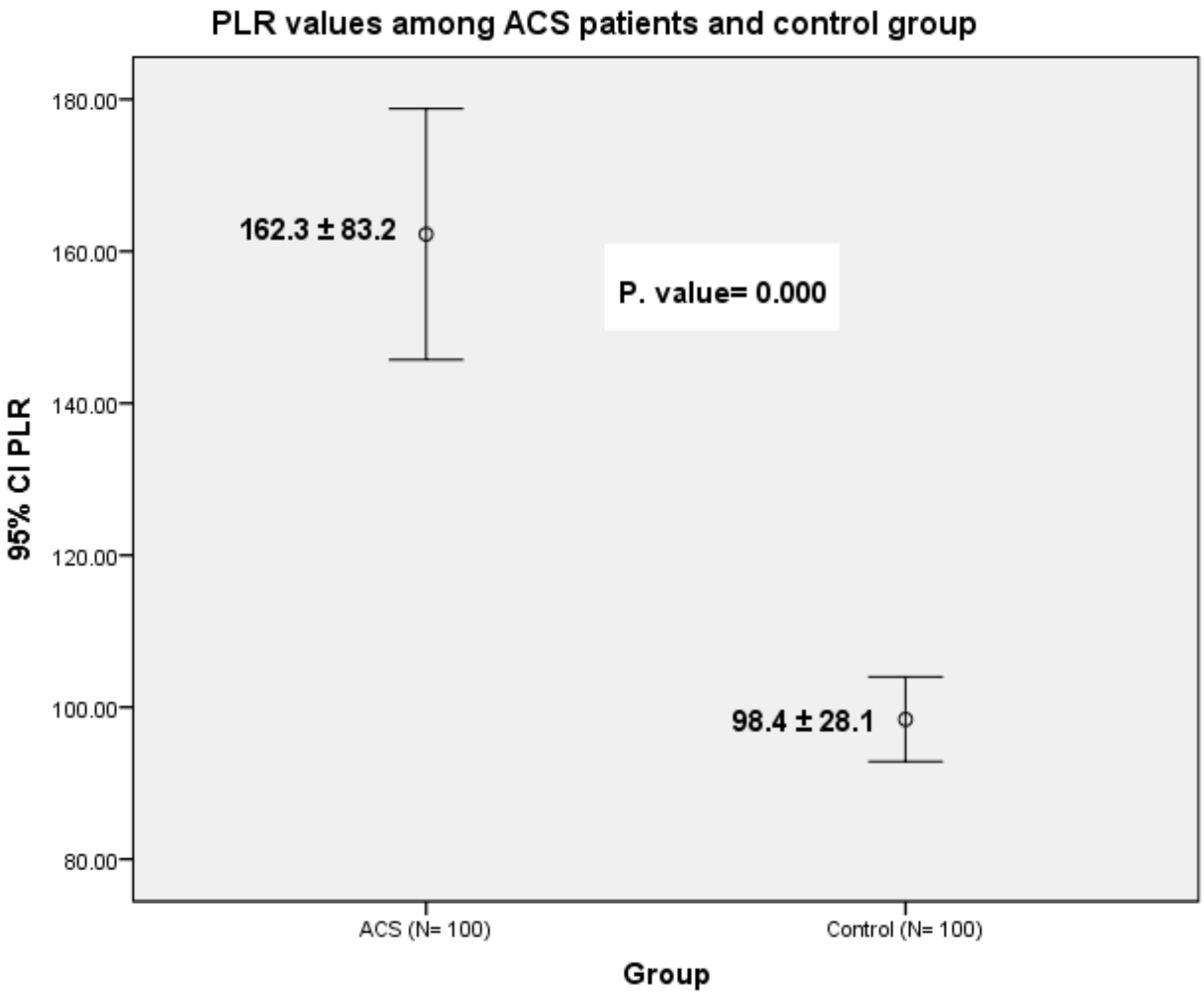

Figure 3: The comparison of PLR values among the case and control groups

Table 3: The mean ± SD of NLR and PLR regarding the age of the case group (N= 100)

	Age (Years)			
	<40	**40-60**	**>60**	**P. value**
NLR	5.2±1.2	5.4±4.4	6.5±5.6	0.729
PLR	129.7±58.5	136.3±65.4	141.7±94.6	0.40

ANOVA test was used

Table 4: The mean ± SD of NLR and PLR regarding the gender of the case group (N= 100)

	Gender		
	Male	**Female**	**P. value**
NLR	6.7±4.7	3.9±2.4	0.008*
PLR	182.5±62.2	156.6±67.4	0.013*

ANOVA test was used

*P. value id significant at level 0.05

Table 5: The mean of NLR and PLR regarding the CVD types among the case group (N= 100)

	CVD types			
	STEMI	**Non-STEMI**	**Unstable angina**	**P. value**
NLR	3.0±0.9	6.8±5.9	2.9±1.8	0.003*
PLR	175.0±73.1	219.0±126.1	147.3±68.3	0.007*

ANOVA test was used

*P. value id significant at level 0.05

Table 6: The comparison of CBC findings among the case and control groups

CBC	**Group**		**P. value**
	CVD (N= 100) Mean (SD)	**Control (N= 100) Mean (SD)**	
Hemoglobin (g/dl)	10.6(1.6)	14.4(2.5)	0.000*
PCV (%)	32.4(5.3)	40.3(1.8)	0.000*
WBCs (10^3/µL)	8.9(1.9)	5.4(1.0)	0.000*
Platelets (10^3/µL)	316(92.2)	286(50.4)	0.000*
Neut. (10^3/µL)	6.8(1.5)	2.7(0.9)	0.000*
Lymph. (10^3/µL)	1.8(0.8)	2.0(0.5)	0.001*

T-test was used

*P. value is significant at level 0.05

CHAPTER FOUR

DISCUSION, CONCLUSION and RECOMMENDATION

4.1 Discussion

The study showed that, NLR was significantly higher among CVD patients than in normal individuals. There were significant reduction in lymphocytes and elevation in neutrophils among CVD patients ($p< 0.05$), consequently, these was reflected in the NLR was significantly difference. Our findings go in same line with several studies (Imtiaz, *et al.,* 2012; Chen, *et al.*, 2018).

PLR has been proposed to be a pro-thrombotic and inflammatory marker (Wang, *et al.,* 2013). In the present study, PLR was significantly higher among CVD patients than in normal individuals. There were significant reduction in lymphocytes and elevation in platelets count among CVD patients ($p< 0.05$),these was reflected in the PLR was significantly difference. Our findings consistence with many of literatures (Tadeusz, *et al.,* 2015; Harun, 2016, Hilman, *et al.*, 2016, Dong . *et al.,* 2017).

Concerning to ongoing efforts to correlate NLR and PLR with CVD type characteristics, the present study showed that, highest value of NLR and PLR found in non-STEMI patients. Azab, *et al* (2012) reported that higher PLR and NLR values were associated with in non-ST elevated myocardial infarction (non-STEMI) patients. However, in one study conducted by Akpek, *et al* (2012) found that the NLR has been evaluated in various studies of coronary artery disease (CAD), especially STEMI.

Despite there no study associated the relationship between gender with NLR and PLR among CVD, the study stated that, the male patients significantly presented higher NLR and PLR more than females.

The study was evaluate NLR and PLR as a measure of systemic inflammation in , patients with CVD at Khartoum state. Patients tendered to be males (65%) and belonged in age group more than 60 years (52%) when compared to normal individuals, which is similar with previous studies (Hyder, *et a.,* 2016; Taha, *et al.*,2017; Hassan, *et al.*, 2018), this may be related to differences in sex hormones in male and female. Among this study groups, the patients in CVD group were trended to be anemic more than normal subjects. Hemoglobin concentration could affect the cardiovascular system through oxygen supply and blood viscosity (Baskkurt and Meiselman, 2003). Several studies were assessed the association between hemoglobin or hematocrit level and CVD, they indicate a reduction in both hemoglobin and PCV (Moo-Young, *et al* 2013; Durmus, *et al.*, 2015).

The present study revealed that, the patients with CVD had significantly ($P<0.08$) showed higher leukocytes counts than normal subjects. WBCs and their subtypes are remarkable in inflammatory markers in CVD (Horne. et al., 2005). As a result of inflammatory stimulus, leukocytes release many inflammatory cytokines causing distractive effects on the myocardium resulting in decrease left ventricle (LV) function and heart failure (HF) (Baldus, *et al.,* 2003; Reichlin, *et al.,* 2010).These findings were in agreement with Madjid. *et al* (2004) and Asadollahi, *et al* (2010) who reported, that an elevation of white blood cell count (WBC) were associated with an increased risk for developing CAD.

In the current study the neutrophil counts were significantly higher in CVD group when compared with normal population. Previous studies reported that high

neutrophil counts are associated with an higher incidence of coronary disease (Adamsson, *et al.*, 2012), heart failure (HF) (Pfister.*et al* 2012), and stroke (Zia, *et al.,* 2012).

The present results demonstrated that there was a significant increase in platelet counts among CVD group in contrast to normal individuals. Increased platelet counts and platelet activation have an important role in thrombus formation and the progression of atherosclerosis. The change of platelets count responding to abnormal vessel walls, can result in arterial thrombosis. Furthermore, increased platelets counts have been demonstrated to promote inflammation and lead to a more aggressive course of active atherosclerosis (Gary, *et al.,* 2013). A previous clinical study has determined that higher baseline platelet counts may be associated with CVD events (Thaulow, *et al.,* 1991). Moreover, Würtz, *et al.*, (2012) reported that, Platelet activation is also significantly associated with platelet counts (Würtz, *et al.*, 2012). The current results in agreement with Li. *et al* (2014) who mentioned that Inappropriate platelet activation is an important pathogenic component of thrombosis at the site of vascular injury and leads to CVD mainly ACS.

The present study demonstrated that, the lymphocytes count was significantly reduced in CVD patients when compared to normal participants. A low blood lymphocyte count has been shown to be related with worse cardiovascular consequences in patients with CAD and chronic heart failure. This can be justified by in cases of sustained inflammation, lymphocyte counts decrease due to increased lymphocyte apoptosis. The present data in accordance with previous studies have demonstrated that relative and absolute lymphocyte concentrations are lower in patients who suffered from CVD (Acanfora, *et al* 2001; Suzuki, *et al.*, 2013).

Although the difference was not significant (P> 0.05) the older CVD patients tended to have the highest value of NLR and PLR. The result is disagreement with Umesh, *et al* (2008)who reported that patients with higher NLRs were significantly older (P= 0.001) and tended to have a lower frequency of previous cardiovascular disease.

4.2 Conclusion

The present study concluded that, the NLR and the PLR of CVD patients were higher than those of the normal individuals. Moreover, both parameters (NLR and PLR) were significantly high in male non-STEMI CVD subtypes.

Neutrophil-lymphocyte ratio (NLR), a new addition to the long list of markers, is an inexpensive, easy to obtain, widely available marker of inflammation, which can aid in the risk stratification of patients with various cardiovascular diseases in addition to the traditionally used markers.

NLR and PLR,is a simple indicator, can initially introduced into clinical practice to improve the diagnosis and prognosis of CVD.

4.3 Recommendations

Further studies with large sample size are needed to assess the role of NLR and PLR to predict outcomes and mortality during the follow-up of Sudanese CVD patients.

References

Acanfora, D., Gheorghiade, M., Trojano, L. et al.(2001). Relative lymphocyte count: a prognostic indicator of mortality in elderly patients with congestive heart failure. *Am. Heart J*., **142: 167-73.**

Adamsson, S., Smith, J.G and Melander, O.(2012). Incidence of coronary events and case fatality rate in relation to blood lymphocyte and neutrophil counts. *Arterioscler.Thromb.Vasc .Biol*., **32:533–539.**

Akpek, M., Kaya, M.G., Lam, Y.Y., Sahin, O., Elcik, D., Celik, T. et al.(2012). Relation of neutrophil/lymphocyte ratio to coronary flow to in-hospital major adverse cardiac events in patients with ST-elevated myocardial infarction undergoing primary coronary intervention. *Am. J .Cardiol.,* **110(5):621-7.**

Alexandrakis, M.G., Passam, F.H., Moschandrea, I.A., Christophoridou, A.V., Pappa, C.A., Coulocheri, S.A. et al.(2003). Levels of serum cytokines and acute phase proteins in patients with essential and cancer-related thrombocytosis. *Am. J .Clin.Oncol*., **26(2):135-40.**

Asadollahi, K., Beeching, N.J and Gill, G.V. (2010). Leukocytosis as a predictor for non-infective mortality and morbidity. *QJM.,* **(103):285–292**

Azab, B., Shah, N., Akerman, M. and McGinn, J.T.(2012). Value of platelet/lymphocyte ratio as a predictor of all cause mortality after non ST elevation myocardial infarction. *J .Thromb.Thrombolysis.,* **34(3): 326-34.**

Bain, B.J., Bates, I., Laffa, M.A and Lewis, S.M. (2011). Dacie and Lewis Practical Haematology,11thed, Churchill Livingstone, *China.*, **p 3-24-47.**

Baldus, S., Heeschen, C., Meinertz, T., Zeiher, A.M., Eiserich, J.P., Munzel, T. et al.(2003). Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. *Circulation.*,**108(12):1440-5.**

Blann, A., Ahmed, N.(2014). Blood Science: Principles and Pathology, 1sted, John Wiely and Sons Ltd, *UK*, **p11, 51.**

Baskurt, O. K., and Meiselman, H. J(2003). Blood rheology and hemodynamics. Semin. Thromb. Hemost. 29, 435-450

Bovill, E.G., Bild, D.E., Heiss, G., Kuller, L.H., Lee, M.H., Rock, R. et al.(1996). White blood cell counts in persons aged 65 years or more from the Cardiovascular Health Study. Correlations with baseline clinical and demographic characteristics. *Am. J. Epidemiol.*,**143:1107–1115.**

Chauhan, S.(2013). An overview on blood purifier, *International Research Journal of pharmacy.*, **4(9): 8-10.**

Cheesbrough, M.(2006). District laboratory practice in tropical countries,2nded, Cambridge University Press, *New York.*,**p 272,297,313-324.**

Ciesla, B.(2012). Hematology in practice, 2ed, F.A.Davis Company, USA., **p 25.**

Colditz, G.A., Willett, W.C. and Stampfer, M. J.(1987). Menopause and the risk disease of coronary disease in women. *New Engl J Med* ., **316:1105-1110.**

Deutsch, V.R and Tomer, A. (2006). Megakaryocyte development and platelet production, *British Journal of Haematology.*, **134(5):453–466.**

Chen, C ., Cong, B.L., Wang, M., Abdullah, M., Long, X. et.al. (2018). Neutrophil to lymphocyte ratio as a predictor of myocardial damage and cardiac

dysfunction in acute coronary syndrome patients). *Integrative. Medicine .Research.*,**7, 2 192-199**

Ceren, S., Nazire, B., Yahya, K. and Öznur, K. (2016). Neutrophil-To-Lymphocyte Ratio And Platelet-To-Lymphocyte Ratio In Cardiac And Non-Cardiac Arrest Distinction: A Retrospective Cohort Study. *Acta.Medica.Mediterranea.,* **32: 1617.**

Ciulla, A.P., Lehman, D.C., (2010). Success in Clinical Laboratory Science: Complete Review,4thed, Pearson Education, New Jersey, p236.

D'Adamo, E., Guardamagna, O., Chiarelli, F., Bartuli, A., Liccardo, D., Ferrari, F and Nobili, V. (2015). Atherogenic dyslipidemia and cardiovascular risk factors in obese children . *International. Journal of Endocrinology .,* **912047. doi : 10.1155/2015/912047 . PMC 4309297 . PMID 25663838 .**

Demirkol, S., Balta, S., Unlu, M., Arslan, Z., Cakar, M., Kucuk, U., Celik, T. et.al.(2012). Neutrophil to Lymphocyte ratio in patient with cardiac syndrome X andits Association with carotid intima-media thickness. *Clinical and applied thrombosis/hemostasis.,* **20-10.1177/1076029612467227.**

Dong Z., Guangyao, W., Yan, F., Zhaofei, W and Xiaojun, L. (2017). Platelet to lymphocyte ratio is associated with the severity of coronary artery disease and clinical outcomes of percutaneous coronary intervention in the Chinese Han population. *Exp.Ther. Med.,* **13(2):731–738.**

Durmus, E., Kivrak, T., Gerin, F., Sunbul, M., Sari, I., Erdogan, O.(2015). Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio are predictors of heart failure. *Arq. Bras. Cardiol.,* **105(6):606-13.**

Finegold, J.A., Asaria, P. and Francis, D.P. (2012). Mortality from ischaemic heart disease by country, region, and age: Statistics from World Health Organisation and United Nations . *International Journal of Cardiology*.**,168 (2): 934–945. doi : 10.1016/j.ijcard.2012.10.046 . PMC 3819990 .PMID 23218570 .**

Folsom, A.R., Rosamond, W.D., Shahar, E., Cooper, L.S., Aleksic, N., Nieto, F.J. et al.(1999). Prospective study of markers of hemostatic function with risk of ischemic stroke. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Circulation.*, **100:736–742.**

Folsom, A.R., Aleksic, N., Catellier, D., Juneja, H.S., Wu, K.K.(2002). C-reactive protein and incident coronary heart disease in the Atherosclerosis Risk In Communities (ARIC) study. *Am. Heart. J.*, **144:233–238.**

Frangogiannis, N.G. (2008). The immune system and cardiac repair.*Pharmacol .Res.*, **58:88–111.**

Gary, T., Pichler, M., Belaj, K., Hafner, F., Gerger, A., Froehlich, H., Eller, P., Rief, P., Hackl, G., Pilger, E. and Brodmann, M. (2013). Platelet-to-lymphocyte ratio: A novel marker for critical limb ischemia in peripheral arterial occlusive disease patients. *PLoS .One*.,**8:e67688.**

Ghosal, K. and Bhaltacharyya, M. (2014). Overview of platelet physiology: it's hemostatic and non-hemostatic role in disease pathogenesis, *The Scientific World Journal*.**(8):1-16**

Greer, J.P., Arber, D.A., Glader, B., List, A.F., Means, R.T., Paraskevas, F. and Rodgers, G.M.(2014). *Wintrobe's Clinical Hematology, 13thed, Lippincott Williams and Wilkins, Philadelphia.*, **p 4, 83.**

Gürsoy, O.M., Karakoyun, S., Kalçık, M., Gökdeniz, T., Yesin, M., Gündüz, S. et al.(2014). Usefulness of novel hematologic inflammatory parameters to predict prosthetic mitral valve. *Am. J .Cardiol*.,**113(5): 860-4.**

Gibson, P.H., Croal, B.L., Cuthbertson, B.H., Small, G.R., Ifezulike, A.I., Gibson, G. et al.(2007). Preoperative neutrophil-lymphocyte ratio and outcome from coronary artery bypass grafting. *Am. Heart J.,* **154:995–1002.**

Harun, K. (2016): The role of platelet-lymphocyte ratio in the severity of coronary artery disease assessed by the angiographic Gensini score. *Anatol. J. Cardiol*., **16(3): 224.**

Hassan, H., Elbagire, A. and Idriss, H. (2018). Clinical and sociodemographic characteristics of cardiovascular disease in Sudan. *PolAnn.Med*., **25(1):11–16.**

Highlander, P. and Shaw, G. P. (2010). Current pharmaco therapeutic concepts for the treatment of cardiovascular disease in diabetics. *Ther. Adv. Cardiovasc Dis.,* **4: 43-54.**

Hillman, R.S., Ault, K.A., Leporrier, M. and Rinder, H.M., (2010). Hematology in Clinical Practice, 5thed, The McGraw-Hill Companies, United State., **p 248-250.**

Hilman, Z., Lukman, Z. and Firman, Z. (2016). Link between the Platelet-to-Lymphocyte Ratio and Coronary Artery Disease Severity. *J. Tehran. Heart Cent*., **13; 11(2): 104.**

Hoff brand., A.V., Moss., P.A.H. (2016). Hoffbrand's Essential Haematology, 7thed, John Wiley and Sons Ltd, UK, **p2,4,90.**

Horckmans, M., Ring, L., Duchene, J., Santovito, D., Schloss, M.J., Drechsler, M. et al.(2017). Neutrophils orchestrate post-myocardia linfarction healing by polarizing macrophages towards are parative phenotype. *Eur. Heart J.*, **38:187–97**

Horne, B., Anderson, J., John, J., Weaver, A. et al. (2005). Which white blood cell subtypes predict increased cardiovascular risk? *J. Am. Coll. Cardiol.*,**45: 1638-1643.**

Howard., M.R. and Hamilton., P.J. (2013). Hematology: An Illustrated Colour Text,4thed*Churchill Livingstone.*, **p 7.**

Hyder, O., Mohammed, A., Akasha, M and Abdulateef S. (2016). Gender in equality in acute coronary syndrome patients at Omdurman Teaching Hospital, Sudan. *J Family Community Med.*,**23(2): 100–104.**

Imtiaz, F., Shafique, K., Mirza, S.S., Ayoob ,Z., Vart, P. and Rao S. (2012). Neutrophil lymphocyte ratio as a measure of systemic inflammation in prevalent chronic diseases in Asian population. *Int. Arch.Med.*, **5(1): 2.**

Jani, B. and Rajkumar, C. (2006). Ageing and vascular ageing .*Postgrad. Med. J* ., **82 (968): 357–362. doi : 10.1136/pgmj.2005.036053 . PMC 2563742 .**

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. and Forman, D.(2011). Global cancer statistics. *CA Cancer J Clin*., **61:69–90.PubMed View Article Google Scholar**

Jilma, B., Blann, A., Pernerstorfer, T., Stohlawetz, P., Eichler, H., Vondrovec, B. et al. (1999). Regulation of adhesion molecules during human endotoxemia. *Amer. J. RespCrit. Care. Med.*,**159(3): 857-63.**

Jousilahti, V. and Tuomilehto, P. (1999). Sex, Age, Cardiovascular Risk Factors, and coronary heart disease. *Circulation.,* **99: 1165-1172.**

Karakas, M., Korucuk ,N., Tosun, V., Altekin, R., Koc, F., Ozbek,S.C. et al.(2016). Red cell distribution width and neutrophil-to-lymphocyte ratio predict left ventricular dysfunction in acute anterior ST-segment elevation myocardial infarction. *J. Saudi Heart Assoc*., **28:152–8.**

Kathiresan, S. and Srivastava, D.(2012). Genetics of human cardiovascular disease. *Cell.,* **148 (6): 1242–57.**

Kawthalkar, S . M. (2013). Essential of Hematology, 2nd ed, *Jaypee Brothers Medical Publisher Ltd.,* **p 2, 32.**

Kolaczkowska, E. and Kubes, P. (2013). Neutrophil recruitment and function in health and inflammation. *Nat. Rev. Immunol.*, **13:159–75.**

Kvan, E., Pettersen, K.I., Sandvik , L. and Reikvam, A. (2007). High mortality in diabetic patient with acute myocardial infarction: cardiovascular co-morbidities contribute most to the high risk. *Int. J. Cardiol.,* **121 (2): 184–188. Doi:10.1016/j.ijcard.2006.11.003 .**

Kwon, H., Kim, S., Oh, S., Lee, S., Lee, J., Choi, H.J. et al.(2012). Clinical significance of preoperative neutrophil lymphocyte versus platelet lymphocyte ratio in patients with operable colorectal cancer. *Biomarkers.,* **17(3):** ***216-22*.**

Lemer, D. J. and Kannel, W.B. (1986). Patterns of coronary heart diseases morbidity and mortality in the sexes: a 26-year follow- up of the Framingham population. *A.m. Heart .J.,* **11:383-390**

Li, W., Gigante, A., Perez, M., Yue, H., Hirano, M., McIntyre, T. and Silverstein, R.L. (2014). Thymidine phosphorylase participates in platelet signaling and promotes thrombosis. *Circ. Res*., **115:997–1006.**

Li, W. et al. (2016). Platelet to lymphocyte ratio in the prediction of adverse outcomes after acute coronary syndrome: a meta-analysis. *Sci. Rep***., 7, 40426;***doi*: **10.1038**

Li-yuan, S., Eun-whan, L., Aqeela, Z and Jae-hyun, P.(2015). Risk Factors of Cardiovascular Disease and Their Related Socio-Economical, Environmental and Health Behavioral Factors: Focused on Low-Middle Income Countries- A Narrative Review Article. *Iran. J. Public. Health*., **44(4): 435–444.**

Lokwani, D.P.(2013). The ABC of CBC: Interpretation of Complete Blood Count and Histogram, 1sted, *Jaypee. Brothers. Medical.Publisher.*, **p 3, 6, 14.**

Mackay., Mensah., Mendis. et al.(2004): The Atlas of Heart Disease and Stroke. *World Health Organization.*

Macrae, Calum, A., Vasan and Ramachandran, S. (2016): The Future of Genetics and Genomics: Closing the Phenotype Gap in Precision Medicine. *Circulation.* **133**

Madjid, M., Awan, I., Willerson, J. and Casscells, S.(2004). Leukocyte count and coronary heart disease: implications for risk assessment. *J. Am.Coll.Cardiol*., **44: 1945–1956.**

McGill, H., McMahan, C. and Gidding, S. (2008). Preventing heart disease in the 21st century: implications of the Pathobiological Determinants of Atherosclerosis inYouth(PDAY)study.*Circulation***.,117(9):121627.***doi:***10.1161/CIRCULATION AHA.107.717033**.*PMID***18316498** .

McPhee and Stephen. (2012): Current medical diagnosis & treatment .*McGraw-Hill Medical*. **p. 430.(25): 2634–39.**

Medicinewise. (2011). NPS Prescribing Practice Review 53: Managing lipids . Archived from the original on 19 March 2011. Retrieved 1 August 2011.

Mehta, A.B., Hoff brand, A.V., (2014). Hematology at a Glance, 4thed,Wiley-Blackwell, London, 199, p147.

Mendelsohn, M.E., Karas, R.H. (1999). The protective effects of estrogen on the cardiovascular system. *New Engl J Med* ., **340:1801–1811.**

Moscai, A.(2013). Diverse novel functions of neutrophils in immunity, inflammation and beyond, *The Journal of Eoerimental Medicine.*, **210(7): 1283-1299.**

Nikpay, M., Goel, A., Won, H., Hall, L. M., Willenborg, C., Kanoni., Stavroula, S., Danish, K., Theodosios, N. and Christopher, P. (2015): A comprehensive 1000 Genomes–based genome-wide association meta-analysis of coronary artery disease. *Nature Genetics* .**47 (10): 1121–1130**. *doi***: 10.1038/ng.3396 .PMC 4589895 . PMID 26343387 .**

Norhammar, A., Malmberg, K., Diderhol, E., Lagerqvist, B., Lindahl, B., Ryde. et al. (2004). Diabetes mellitus: the major risk factor in unstable coronary artery disease even after consideration of the extent of coronary artery disease and benefits of revascularization. *J. Am. Coll.Cardiol* ., **43(4):585–591.doi: 10.1016/j.jacc.2003.08.050** .

Ong, S.B., Hernández-Reséndiz, S., Crespo-Avilan, G.E., Mukhametshina, R.T., Kwek, X.Y., Cabrera-Fuentes, H.A. et al.(2018). Inflammation following acute

myocardial infarction multiple players, dynamic roles, and novel therapeutic opportunities. *Pharmacol.Ther.*, **98:111-119.**

Palis, J. (2014). Primitive and definitive erythropoiesis in mammals. *Frontiers in Physiology*, **5(1664-042x):1-28.**

Pfister, R., Sharp, S.J. and Luben, R.(2012). Differential white blood cell count and incident heart failure in men and women in the EPIC-Norfolk study. *Eur. Heart J.*, **33:523–530.**

Prabhu, S.D. and Frangogiannis, N.G.(2016). The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis. *Circ.Res.*, **119:91–112.**

Rozenberg, G., (2011). Microscopic Hematology: a practical guide for the laboratory, 3rded. *Churchill. Livingstone.*, **p46, 92,106.**

Ruparelia, N., Chai, J.T., Fisher, E.A. and Choudhury, R.P.(2017). Inflammatory processes in cardiovascular disease: a route to targeted therapies. *Nat.Rev. Cardiol*., **14:133–44.4.**

Shanthi, Mendis., Pekka, Puska., Bo, Norrving., World Health Organization (2011). *Global Atlas on Cardiovascular Disease Prevention and Control (PDF)*. World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization. **pp. 3–18 (PDF) from the original on 2014-08-17.**

Song, Yue., Jiahua, Zhang., Jingyang, Wu., Weiping,Teng., Lei, Liu., Lei, Chen. J., Environ and Res. (2015): *Public Health.***12, 10009-10019.**

Stephenson, E., Savvatis, K., Mohiddin, S.A. and Marelli-Berg, F.M.(2017). T-cell immunity in myocardial inflammation pathogenicrole and therapeutic manipulation. *Br. J. Pharmacol.,* **174:3914–25.**

Suliman, A. (2011). The state of heart disease in Sudan. *Cardiovasc.J. Afr.*, **22(4):191-6.**

Suzuki, T., FutamiSuda, S., Igari, Y., Watanabe, K., Ouchi, M., Suzuki, K., Sekimizu, K., Kigawa, Y., Nakano, H. and Oba, K. (2013). Low-molecular-weight lipoprotein (a) and low relative lymphocyte concentration are significant and independent risk factors for coronary heart disease in patients with type 2 diabetes mellitus: Lp(a) phenotype, lymphocyte, and coronary heart disease. *Lipids. Health. Dis.,* **12:31.**

Tadeusz, O., Jarosław, W., Andrzej, L., Joanna, S. et al. (2015). The platelet-to-lymphocyte ratio as a predictor of all-cause mortality in patients with coronary artery disease undergoing elective percutaneous coronary intervention and stent implantation. *J. Saudi. Heart. Assoc.,* **27(3): 144–151.**

Taha, A. and Mirghani, H. (2017). Basic Clinical Characteristics and Hospital Outcomes of cardiovascular disease Patients – Sudan. *Sudan Journal of Medical Sciences (SJMS).,* **12(2):52-62**

Tang, T.T., Yuan, J., Zhu, Z.F., Zhang, W.C., Xiao, H., Xia, N. et al.(2012). Regulatory T cells ameliorate cardiac remodeling after myocardial infarction. *Basic. Res. Cardiol.,* **107:232.**

Temiz, A. (2014). Platelet/lymphocyte ratio and risk of in-hospital mortality in patients with ST elevated myocardial infarction. *Medical science monitor: international medical journal of experimental and clinical research.,* **20:660–665**

Thaulow, E., Erikssen, J., Sandvik, L., Stormorken, H. and Cohn, P.F. (1991). Blood platelet count and function are related to total and cardiovascular death in apparently healthy men. *Circulation.,* **84:613–617.**

Tozawa, K., Ono-Uruga, Y., Matsubara, Y., (2014). Megakaryopoiesis, *Clinical and Experimental Thrombosis and Hemostasis*, 1(2):54-58.

Turgeon, M.L.(2012). Clinical Hematology: Theory and Procedures, 5thed, Lippincott Williams and Wilkins., **p73, 79.**

Ugur, M. (2014). The relationship between platelet to lymphocyte ratio and the clinical outcomes in ST elevation myocardial infarction underwent primary coronary intervention. Blood coagulation & fibrinolysis*: an international journal in haemostasis and thrombosis*., **25: 806–811**

Umesh, U., Sanjay, A., Daniel, M., Kim, A. and Hitinder, S. (2008). Association Between Admission Neutrophil to Lymphocyte Ratio and Outcomes in Patients With Acute Coronary Syndrome. *A.J.Conline.*,**653-657**

Vanhecke, T. E., Miller, W. M., Franklin, B. A., Weber, J. E. and McCullough, P. A.(2006): Awareness, knowledge, and perception of heart disease among adolescents. *Eur. J. Cardiovasc Prev. Rehabil.*,**13 (5): 718-723.**

Wang, D., Yang, J.X., Cao, D.Y., Wan, X.R., Feng, F.Z., Huang, H.F, et al. (2013). Preoperative neutrophil-lymphocyte and platelet-lymphocyte ratios as independent predictors of cervical stromal involvement in surgically treated endometrioid adenocarcinoma. *Onco. Targets. Ther.*,**6: 211-6.**

WHO.(2016). Cardiovascular diseases (CVDs). Available at: *http://www.who.int/mediacentre/factsheets/fs317/en/*

Wingard, D.L., Suarez, L. and Barrett-Connor, E. (1983). The sex differential in mortality from all causes and ischemic heart disease. *Am. J. Epidemio.*,**1117:165-172**

Witteman, J.C., Grobbee, D.E., Kok, F.J., Hofman, A. and Valkenburg, H.A. (1989) Increased risk of atherosclerosis in women after the menopause. *B.M.J* ., **298:641-644**

Würtz, M., Hvas, A.M., Kristensen, S.D. and Grove, E.L. (2012). Platelet aggregation is dependent on platelet count in patients with coronary artery disease. *Thromb. Res.*, **129:56–61.**

Zhou, D. (2016). Platelet-to-Lymphocyte Ratio Improves the Predictive Power of GRACE Risk Score for Long-Term Cardiovascular Events in Patients with Acute Coronary Syndrome. *Cardiology.*, **134: 39–46.**

Zia, E., Melander, O. and Bjorkbacka, H. (2012). Total and differential leukocyte counts in relation to incidence of stroke subtypes and mortality: a prospective cohort study. *J. Intern. Med*., **272:298–304.**

Zlatanova, I., Pinto, C. and Silvestre, J.S. (2016). Immune modulation of cardiac repair and regeneration: the art of mending broken hearts. *Front. Cardiovasc. Med.*, **3:40.**