**ADDIS ABABA UNIVERSITY**
**COLLEGE OF HEALTH SCIENCE**
**SCHOOL OF MEDICINE**
**DEPARTMENT OF MEDICAL BIOCHEMISTRY**

# Cross sectional comparative study of hematological parameters in people withatherosclerotic cardiovascular disease and those with cardivascular risk alone.

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**A Thesis submitted to the school of graduate studies of Addis Ababa University for partial fulfillment of the requirements for the degree of Master of Science in Medical Biochemistry**

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# ADDIS ABABA UNIVERSITY

## School of Graduate Studies

## Department of Medical Biochemistry

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This is to certify that this Masters research thesis entitled: "**Cross sectional comparative study of hematological parameters in people withatherosclerotic cardiovascular disease and those with cardivascular risk alone"** a cross sectional comparative study conducted by **Ebsitu Abate** and Submitted to the Department of Medical Biochemistry for partial fulfilment of the requirements for the degree of Master of Science in Medical Biochemistry complies with the regulation of the university and meets accepted standards with respect to originality and quality.

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## ABBREVIATIONS AND ACRONYMS

**ACE:** Angiotensin Converting Enzyme

**AS:** Aldosterone Synthase

**ASCVD:** Atherosclerotic cardiovascular disease

**BP:** Blood Pressure

**CHD:** Coronary Heart Diseases

**CRP:** C - reactive protein

**CVD:** Cardiovascular Disease

**DOB:** department of biochemistry

**Hct:** Haematocrit

**Hgb:** Haemoglobin

**hsCRP:** Highly Sensitive C - reactive protein

**HTN:** Hypertension

**IHD:** Ischemic heart disease

**IR:** Insulin Resistance

**MCH:** Mean Corpuscular Haemoglobin

**MCHC:** Mean Corpuscular Haemoglobin Concentration

**MCV**: Mean Corpuscular Volume

**MetS**: Metabolic Syndrome

**MPV**: Mean Platelet Volume

**NADH:** Nicotinamide Adenine Dinucleotide (Reduced form

**NADPH**: Nicotinamide adenine dinucleotide phosphate (reduced form)

**NCD:** Non Communicable Disease

**NF-κB**: Nuclear Factor κB).

**NLR**-Neutrophil Lymphocyte Ratio

**NO:**Nitric Oxide

**PDW:** Platelet Distribution Width

**PKC:** Protein Kinase C

**PLR** -Platelet Lymphocyte Ratio

**PLT**: Platelet

**PPARγ:** Peroxisome proliferator-activated receptor gamma

**RAAS**: Rennin Angiotensin Aldosterone System

**RBC:** Red Blood Cells

**RDW**: Red Cell Distribution Width

**ROS:** Reactive Oxygen Species

**SOM:** School of Medicine

**TASH:** TikurAnbessa Specialized Hospital

**WBC:** White Blood Cells

**WHO:** World Health Organization

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## ABSTRACT

**Background**

Cardiovascular disease (CVD) is the foremost killing disease worldwide and ASCVD is one of the prominent types of CVD which is mainly associated with a condition called atherosclerosis. Various risk factors are associated with its incidence. These are hypertension, diabetes, dyslipidaemia, smoking, genetic factors etc. Presence of ASCVD as well as its risk factors results in disturbance of various physiological and biological function of the body. Haematological parameters for instance tend to be disturbed in the presence of abnormal physiological and biological function.

**Aim:** Therefore, in this prospective cross sectional comparative study we have assessed and compared the pattern of haematological parameters in peoples having established atherosclerotic cardiovascular disease (ASVD) vs. peoples having ASCVD risks alone who attended TASH Addis Ababa, Ethiopia and also correlated haematological parameters with the novel inflammatory marker hsCRP.

**Method:** -Blood samples were collected from study participants and serum was extracted for the lipid and hsCRP analysis and the remaining whole blood is used for haematological parameters determination using SYSMEX machine while Roche/Hitachi Cobas 6000 analyser that uses immunoturbidimetric assay was used to determine hsCRP whereas lipid profile was first determined by spectrophotometric analysis and then repeated by using Roche/Hitachi Cobas 6000 analyser.

The total number of participant was**100 (50 in each group)**.

**Result: -**White blood cells (*WBC), mean cell volume (MCV), red cell distribution width (RDW) and monocyte (MONO)* are significantly higher in pre-existing ASCVD and also have significant association with the presence of ASCVD. Mean platelet volume (*MPV)* significantly higher in ASCVD risked group and associated with the presence of the risk. Additionally, in correlation analysis of highly sensitive C-reactive protein (hs*CRP)* with hematological parameter h**s***CRP*s show significant correlation with MPV. It is higher and significantly associated with the presence of risk factors as well.

**Conclusion: -**Using this affordable, routinely tested and easily available tests may help to infer future ASCVD risk as well as the presence of ASCVD morbidity while hsCRP level in comparison group vs. cases require further study.

# 1. INTRODUCTION

## 1.1 BACKGROUND

Cardiovascular disease is the foremost reason for many deaths worldwide which is accountable for around 17·8 million deaths in 2017 (Kaptoge, *et al.,* 2019) and atherosclerotic cardiovascular disease (ASCVD) is one of the most epidemic types of CVD which is becoming a huge burden in the population (Barquera, *et al*., 2015).

ASCVD is related with a condition called *atherosclerosis,* deposition of fat in the artery due to endothelium dysfunction, which commonly manifested as *coronary heart disease* and *stroke* (Barquera, *et a*l., 2014).

Various factors are indicated as risk factors for atherosclerosis and its complication but the conventional risk factor that accounts between 70% and 90% are hypertension, diabetes, dyslipidaemia, aging, cigarettes smoking, alcohol consumption, unhealthy diet and lack of physical exercise (Vinereanu,2007). The presence of metabolic syndrome (Mets) also indicated as the other risk factor (Ninomiya *et al.,* 2004). The main component of this MetS involves obesity, insulin resistance, dyslipidaemia, and hypertension (Cornier *et al.*, 2008).

Hypertension is defined as systolic/diastolic blood pressure above 140/90 mm Hg. *Systolic pressure* is the highest pressure in the artery when the heart beats and fills the artery while *diastolic pressure* is the lowest pressure in the artery when the heart relaxes between beats (Kaplan, 2010).

Diabetes is a group of chronic metabolic diseases characterized by hyperglycaemia as a result of defects in insulin secretion, insulin action, or both (American Diabetes Association, 2010). Both hypertension and diabetes are known to be substantial risk factor for macro vascular and micro vascular complications, including atherosclerotic cardiovascular disease (ASCVD) and peripheral vascular disease, retinopathy, nephropathy, and possibly neuropathy (American Diabetes Association, 2003; Fowler, 2008; ELLULU, *et al*., 2016).

Various metabolic disease including cardiovascular diseases are known to be associated with haematological parameters that can be used as a marker for vascular complications (Nebeck *et al.,* 2012). For instance, haemoglobin, haematocrit, white blood cell (WBC), red blood cell (RBC) and blood platelet (PLT) counts level are

elevated in association to MetS in adults (Aypak *et al.,* 2014), increased formation of platelet-leukocyte conjugates in MetS contribute to the pathophysiological processes that will increase risk for atherosclerotic disorders (Harrison *et al*., 2005; Arteaga *et al.*, 2006; Lohsoonthorn *et al.*, 2007) and RDW also indicated to be associated with increased risk of mortality and CVD events in patients with established coronary artery diseases (CAD).

In patients with metabolic disorders there are increased inflammatory markers (e.g. C-reactive protein, chemokine's, adhesion molecules, etc.) (Stefanadi *et al.,* 2010) and *C-reactive protein* is the novel inflammatory biomarker that is elevated promptly during inflammation (Laaksonen *et al.,* 2004).

C- reactive protein (CRP); a serum protein which was discovered >80 years ago and it is also known to be associated with metabolic diseases (Timpson *et al.,* 2005). Since it binds to C polysaccharide of *Streptococcus pneumonia* it is called CRP (Carlson *et al.,* 2005). It is proven to be valuable determinant of disease progression and effectiveness of treatments for a number of medical conditions like, cancer, diabetes, infection, and inflammation (Stone and Kazil, 2014).

Very few studies are presented on the evaluation of haematological parameters in peoples with atherosclerotic cardiovascular disease or in thus with risk alone without cardiovascular diseases. However, there is a gap of information on the associations of haematological parameters of peoples with atherosclerotic cardiovascular disease in contrast with ASCVD risk alone. Therefore, the aim of this study was to compare complete blood cell counts of peoples with atherosclerotic cardiovascular disease and thus with risk alone without ASCVD and correlate it to CRP that to describe as cost effective indicator of increased risk for ASCVD or morbidity of ASCVD.

## 1.2 LITREATURE REVIEW

### 1.2.1 Atherosclerotic cardiovascular disease (ASCVD)

Cardiovascular disease, disorder of heart and blood vessels, is one of the dire but preventable diseases universally which is becoming the foremost cause of death in the world (Wilkins *et al*., 2017). Based on their cause and clinical characteristics there are various types of CVD. Among them the one which is related to a condition named as

atherosclerosis (slow deposition of lipids predominantly cholesterol and its esters in the intima of large pliable and muscular artery) called atherosclerotic cardiovascular disease (ASCVD) (Frostegård, 2013).

Atherosclerotic cardiovascular disease (ASCVD) is the type of CVD that almost take the first rank as a cause of mortality globally (Barquera *et al*., 2015). It encompasses two most commonly occurring disease; Coronary heart disease and cerebrovascular disease (ischaemic stroke) (Barquera *et al.*, 2014).

*Coronary heart disease* which is also called ischaemic heart disease is the result of obstruction of coronary artery due to the deposition of lipids following injury or infection (Chlamydia pneumonia, human cytomegalovirus) that disturb the normal endothelial function and lead to the migration of WBCs, monocytes, lymphocytes, to endothelium. This result in atherosclerotic lesion ,*fatty streak*, formation that typically consist of macrophages and T cells embedded in a thin layer of lipids on the arterial wall and the release of several pro-inflammatory molecules like IL-6, TNF –α, chemokine, adhesion molecules and growth factors (Libby and Theroux, 2005; Chilton, 2004). This induces formation of atherosclerosis and finally cause reduction of endothelium nitric oxide (NO) synthase with subsequent reduction in NO production which may leads to coronary artery vasoconstriction (Shrivastava *et al*., 2015; Grayston *et al*., 2015; Du *et al.*, 2018).

*Ischaemic stroke* is a vascular disorder that occurs due to the blockage of cerebral artery. It has a very complex pathophysiology; disruption of ion balance ($Na^+$–$K^+$ pump, increase in calcium concentrations that result of raised exocytosis (excessive accumulation of excitatory amino acids))that results in excess generation of nitric oxide (NO) by endothelium NO synthase which in turn brings excess production of free radical and finally oxidative stress with subsequent activation of astrocyte and microglia. This brings the production and release of pro-inflammatory cytokines like interleukin-1 (1L-1), tumour necrosis factor1 (TNF-α), interleukin-1β (1L-1β), neuroprotective factors; such as erythropoietin, TGFb1, and metallothionein-2. All this leads to the impairment of the blood brain barrier (BBB) and reperfusion injury which result in cerebral artery blockage and finally ischaemic stroke (Guo *et al*., 2013; Liand Yang, 2017; Orellana-Urzúa *et al*., 2020).

### 1.2.2 ASCVD risk factors

Major risk factors indicated for ASCVD are obesity, diabetes, hyperlipidaemia, hypertension and smoking (Inoue, 2014). But there are also other factors like stress, genetic, gender, age etc (Balakuma *et al*., 2016)

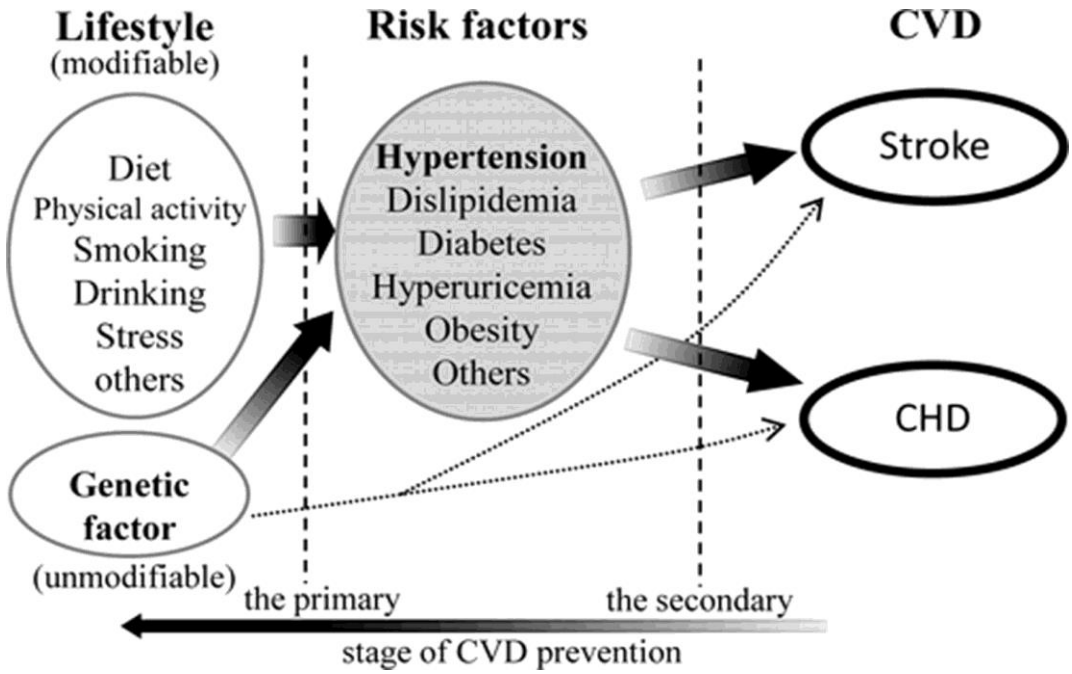


Figure 1. schematic representation of modifiable and unmodifiable ASCVD risk factors (Kokubo, 2014).

Hypertension is a condition of high blood pressure in which the high and long-term force of the blood against the arterial walls may eventually be a major risk factor for almost all types of cardiovascular disease including stroke, coronary heart disease, myocardial infarction, congestive heart failure, etc (Kjeldsen,2018).

Incidence and severity of hypertension depends on various factors like age, life style (diet), and genetic factors. Ageing brings a number of profound changes in the cardiovascular system. Mostly observed changes are gradual dilatation and hardening of the large arteries which has important physiological role in reducing pulse pressure, smoothing peripheral blood flow and improves the efficiency of the cardiovascular system as a whole by buffering the oscillatory changes in blood pressure resulting from intermittent ventricular ejection. Therefore, Stiffening of these arteries leads to a number of adverse hemodynamic consequences, like a widening of pulse pressure and,

eventually, the development of isolated systolic hypertension (McEnier*et al.*, 2007; Schillaci *et al.*, 2007).

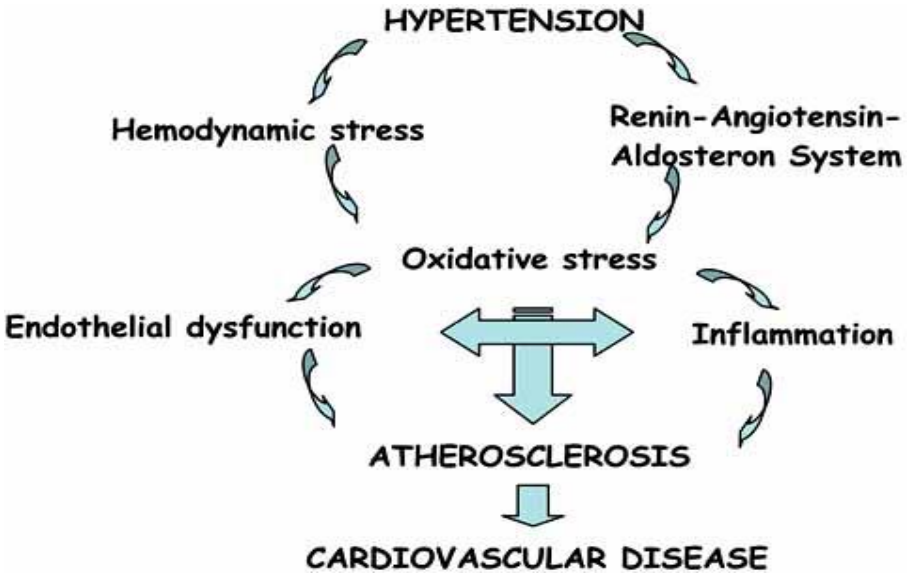


Figure 2. Linkage of hypertension and CVD (Cachofeiro *et al*.,2009)

Diabetes is a condition of chronic hyperglycaemia that occurs due to lack of insulin, resistance of insulin or both. Insulin is a naturally occurring peptide hormone, produced by the beta cells of the pancreas and helps the body use macromolecules for energy (American Diabetes Association, 2010).

Chronic hyperglycaemia in diabetes associated with both macro vascular (cardiovascular disease) and micro vascular complication which is the biochemical process of advanced glycation end products (AGE) which are a complex group of compounds that are formed and deposited irreversibly due to a non-enzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids (Basta *et al*., 2004; Goh and Coope, 2008). AGE can also be derived from diet like animal food products, particularly those processed under high heat and causally involved in insulin resistance (IR) and T2D (Uribarri *et al*., 2015) .

AGE are indicated to have effects on vessels and tissues by binding on receptors like lactoferrin, scavenger receptors types I and II, oligosaccharyl transferase-48 (OST-48), 80K-H phosphoprotein, galectin-3, and CD36. This AGE-RAGE (AGE receptor) interaction activate an array of signal transduction cascades, inflammatory response and ROS-related NF-kB activation and brings up diabetes related complications;

retinopathy, nephropathy, neuropathy,cardiovascular disease etc (Ramasamy *et al*., 2005; Singh *et al*., 2014; Ott *et al*., 2014).

Development of hypertension and diabetes share common pathways that interact and influence each other. These are mental stress and sympathetic nervous system local angiotensin- aldosterone system (RAAS), inflammation and oxidative stress, obesity, adipokines, insulin resistance, and Peroxisome proliferator-activated receptor Gamma (PPAR-γ) (Cheung and Li, 2012).

The mental stress can be chronic or acute. Acute mental stress is resulted from traumatic events and has short duration; it is indicated to cause arterial stiffness by increasing arterial index and also can elevate pulse wave velocity (Kume *et al*., 2021). It is also associated with hypercoagulability state (Zgraggen *et al.,* 2005) While chronic mental stress resulting from the modern lifestyle indirectly linked with the development of diabetes and hypertension frequently which are the major risk factor for ASCVD. But, the mechanism is not well understood however animal experimental study showed that it may be through a mechanism that involve renal sympathetic nerve activity (RSNA) (Barrett *et al.,* 2003; Chapuis *et al*., 2004) and blood pressure control in which baroreflex function is involved (Kanba *et al.,* 2007; McDowall *et al.,* 2006).

Inflammation is a critical causal process strongly related to the initiation, progression and clinical manifestations of cardiovascular disease for which hypertension and diabetes are also risk factors (Androulakis *et al*., 2011). It is the result of the coordinated action of many cell types and mediators that can be acute or chronic (Monteiro and Azevedo, 2010). The chronic inflammation indicated as component of metabolic syndromes (Sutherland *et al*., 2004) which is a result of both innate and adaptive immune systems interaction on two cell types; epithelial cell and a mesenchymal cell of the affected organs. This in turn results in the recruitment of leukocytes, expression of adhesion molecule and inflammatory cytokines like IL-6 IL-1, IL-18, TNF, on the vascular endothelium and brings tissue injury, oxidative stress, remodelling of the extracellular matrix, angiogenesis, and fibrosis in diverse target tissues will result in the development of chronic disease like diabetes and hypertension (Libby, 2007).

Oxidative stress is state of an imbalance between production and inactivation of reactive oxygen species (ROS) which are end products of normal metabolism. Even though ROS have essential role in multiple physiological systems; cell signalling and regulation, they are associated with the pathogenesis of a variety of human diseases, including atherosclerosis, diabetes, hypertension, aging, Alzheimer's disease, kidney disease and cancer (Roberts and Sindhu, 2009). Angiotensin II for instance indicated for generation of vascular inflammation by stimulating NADH/NADPH oxidase, and activates Rho/Rho kinase, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) that induce oxidative stress, resulting in up-regulation of pro-inflammatory transcription factors such as NF-κB (nuclear factor κB).This brings up the generation and secretion of reactive oxygen species ROS, inflammatory cytokines (eg, interleukin-6 [IL-6]), chemokine's, and adhesion molecules) that in turn, results in the generation of inflammatory mediators that lead to endothelial dysfunction and vascular injury and finally result in metabolic diseases (Savoia and Schiffrin, 2007; Jesmin *et al.,* 2010).

Peroxisome proliferator-activated receptor Gamma (PPAR-γ) is nuclear hormone receptor superfamily that has been indicated to have a role in the modulation of critical aspects of development and homeostasis, including adipocyte differentiation, glucose metabolism and macrophage development (Moore *et al*., 2001; Cipolletta *et al*., 2012). It lowers blood pressure, induce favourable effects on the heart, and ameliorate endothelial dysfunction through anti-oxidant, anti-proliferative, anti-hypertrophic, ant fibrotic effects. Thus, the mRNA and protein of PPAR-α and PPAR-γ down regulation by angiotensin II results in the reduction of PPAR anti-inflammatory capacity and activation of inflammation (Madhavan *et al.,* 2006; Shah *et al*.,2008).

Obesity is another factor that predisposes to hypertension, diabetes and CVD. It is defined as BMI greater than or equal to 30 which is measured using weight (kg) divided by heights square (cm). Obesity alters the progression of cardiovascular disease through various mechanisms (Kurukulasuriya *et al.,* 2008); It is linked with hypertension by affecting the pressure-natriuresis relationship that in turn results in enhancement of sympathetic tone, activation of the renin-angiotensin system (RAS), hyperinsulinemia, structural changes in the kidney, and elaboration of adipokines hormones such as leptin and also results in increased renal sodium reabsorption,

impaired pressure natriuresis, and volume expansion (Re, 2009; Landsberg *et al.*, 2013).

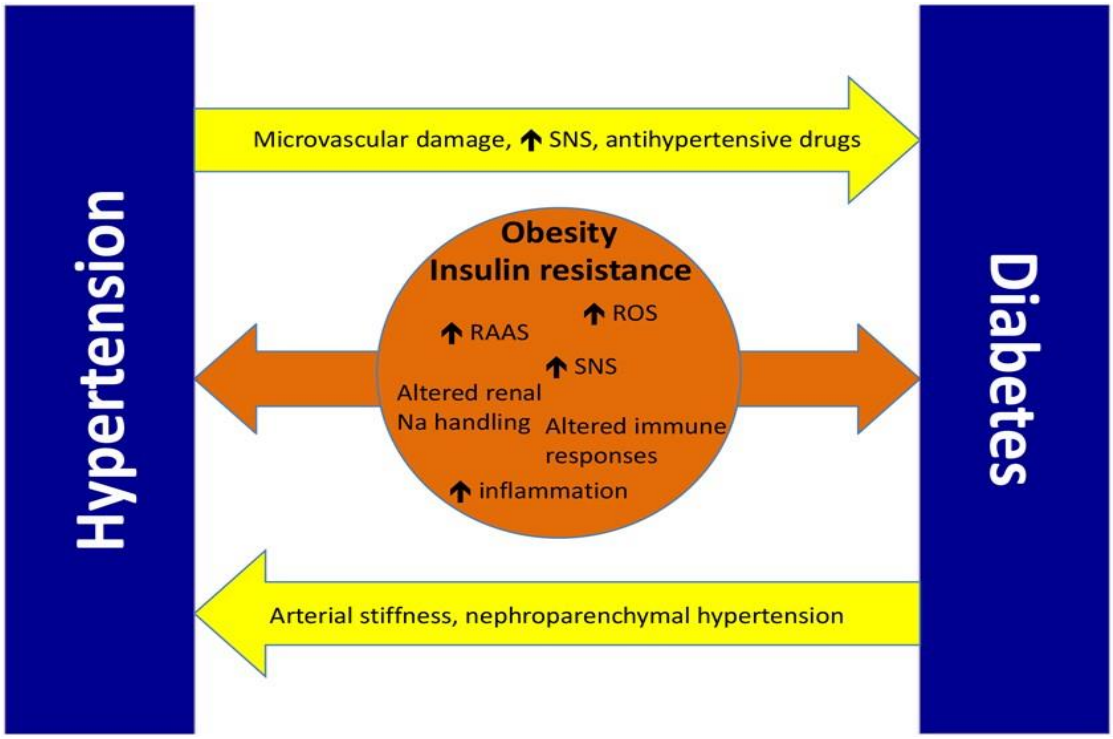


Figure 3. Common pathway of diabetes and hypertension development (Bruno *et al*., 2016).

Hyperlipidaemia (hypercholesterolemia; elevated levels of plasma cholesterol, hypertriglyceridemia; elevated level of triglycerides, or mixed hyperlipidaemia; both, or reduced levels of high density lipoprotein cholesterol (HDL-C) are another metabolic abnormality that act as a risk factor for cardiovascular disease and commonly detected in patients with hypertension, diabetes s and other metabolic syndrome. It is associated with atherosclerosis that results in the development atherosclerotic cardiovascular disease (ASCVD) (Hlaing and Park, 2013; Srikanth. and Deedwania, 2016; Lertwanichwattana *et al*., 2021).

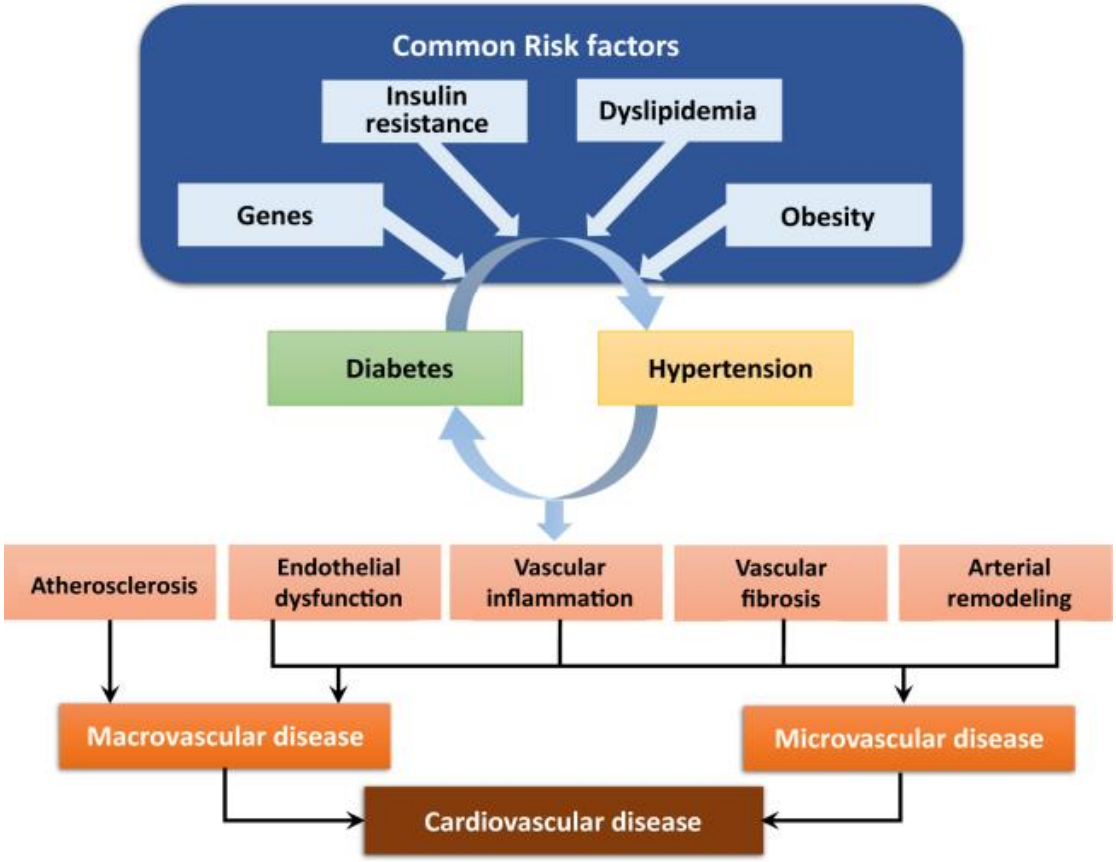


*Figure 4 summary of CVD risk factors and path ways for CVD development* (Petrie, *et al*., 2018)

### 1.2.3 Biomarkers of ASCVD

A number of biomarkers are pointed out for cardiovascular diseases; commonly known biomarkers (lipid profile, glucose, and hormone level), physiological biomarkers (serum ferritin, triglyceride to HDL (high density lipoproteins) ratio, lipophorin-cholesterol ratio, etc), Immunohistochemical biomarkers (Microfiblliar associated protein, CD31), Oxidative Stress-Related Biomarkers, inflammatory biomarkers (hsCRP, chemokine's ….), etc (Yousuf *et al*., 2013; Upadhyay, 2015; Dong *et al* 2019).

Abnormality of *lipid profile* is indicated to be commonly observed in CVD. For instance, Higher LDL cholesterol level, low HDL level, hypertriglyceridemia is indicated to be the major risk factor ASCVD (Nordestgaard, 2016; Peters *et al*., 2016; Barter and Genest, 2019) and also there is an indication of the presence of strong relationship between increased sdLDL (emerging biomarker) level and coronary heart disease risk (Duran *et al.*, 2020). Additionally, study done by Sarwar *et al* indicated

that there is moderate and highly significant relationship between the level of try glyceride and risk of CHD (Sarwar *et al*., 2006)..

C reactive protein is part of acute phase response which increases up to 1000-fold after the onset of inflammation (Ablij and Meinders, 2002). Its level has strong relation with individual components of metabolic syndrome. Increased levels of highly sensitive C-reactive protein (hsCRP) for instance shown to be observed in: upper-body obesity, hypertriglyceridemia, low HDL, hypertension, and diabetes (Ridker *et al.*, 2003). Currently available measurement of CRP assay is called highly sensitive CRP assay. hsCRP level determine the degree of cardiovascular risk factors ; its level less than 1, 1 to 3, and greater than 3 mg/L are associated with lower, moderate, and higher cardiovascular risks, respectively (Bassuk *et al* ., 2004). Various studies like study done in Korea, Japanese, Italy has showed that CRP level is elevated in hypertensive peoples as compared to the normotensive one and suggested that CRP can be an independent risk indicator for hypertension (Chul Sung *et al.,* 2003; Schillaci *et al.,* 2003; Imatoh *et al.,* 2007). There is similar result and suggestions from African countries like sudanes (Tolmay *et al.,* 2012), Nigeria (Idemudia and Idogun, 2012) that showed the positive correlation between atherogenic hsCRP index and CHD risk in hypertensive people. Similarly study done in Ethiopia on diabetic patients with metabolic syndrome who have a risk of developing hypertension indicated that C-reactive protein was strongly associated with serum insulin concentration (Tadesse *et al*., 2014). In addition to the above biomarkers few recent studies tried to indicate the importance of haematological parameters in cardiovascular diseases.

### 1.2.3.1 Haematological parameters

Haematological parameters are parameters that are related to the blood and blood forming organs which are the important biomarkers in our body and used to assess disease progression or response to therapy. They may vary depending on age, gender, race, environmental and genetic background (Etim *et al.*, 2014; Kone *et al.*, 2017). It involves red blood cell indices (mean corpuscular volume, mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration), the red cell distribution width (RDW), white blood cells and platelet count, haemoglobin concentration, haematocrit, and platelet indices (platelet distribution width, PDW, mean platelet volume) (Demir*et al*., 2002; van Kimmenade *et al*., 2010; Kullo *et al*., 2010; Vagdatli *et a*l., 2010; Yuri Gasparyan *et al*., 2011)

#### 1.2.3.1.1 Changes in haematological parameters in the presence of cardiovascular disease and cardiovascular disease risk factor.

WBC is one of blood components that are involved in disease prevention and are also called leucocyte. Its laboratory determination is called leukocyte count or WBC count. Abnormal high WBC count has been positively associated with elevated cardiovascular mortality, mainly due to coronary heart disease and ischemic stroke and considered to be a marker of inflammation related with the initiation and development of atherosclerosis, cardiovascular disease etc (Fan *et al*., 2010; Babio *et al*., 2013; Kim *et al*., 2017;).

The type of WBC that can act as a marker for increased risk of cardiovascular disease has been a subject of several studies. Some studies showed that both high neutrophil and low lymphocyte counts constituted independent predictors of myocardial infarction (Buyukkaya *et al*., 2014).Similarly, other studies found a significant correlation between neutrophil, lymphocyte and eosinophil counts and metabolic syndromes (Kim *et al*.,2008). One study has found that elevated monocyte counts, high neutrophil counts a higher neutrophil lymphocyte ratio and lower eosinophil to be predictive of greater cardiovascular mortality(Horne *et al*., 2005; Waterhouse *et al*., 2008;Verdoia *et al*., 2016 ; Kim, *et al*., 2017; fan *et al*., 2018; Júnior and Cipriano, 2019).

RBCs are 8μm diameter biconcave shape blood cells. Being biconcave provides the red cell with a maximum surface-for-gas exchange as well as optimal deformability. They contain haemoglobin, which carries oxygen throughout our body to all of our tissues and organs and

gives RBC pale red colour. High haemoglobin value is indicated to be associated with risk of ischemic strokes. High RBC count is also demonstrated to be a strong independent predictor of acute cardiovascular events (Choi *et al*., 2003; Munker *et al.,* 2007).

Normally all RBCs have the same colour, size, and shape. However, certain conditions can cause variations that impair their ability to function properly. Size, shape, and physical characteristics of the RBCs are measured by haematological parameters that have significant role in the diagnosis of different disease called RBC indices (Sirdah *et al*., 2015). There are three parts of RBCs indices that were first introduced by Wintrobe in 1929; MCV (the average size of the RBCs constituting the blood), MCH (the average weight of haemoglobin in the RBCs), MCHC refers to the average concentration of haemoglobin in the RBCs contained within the sample (Zandecki, *et al*., 2007; Bordbar *et al*., 2015). These are calculated from haemoglobin, haematocrit, and red blood cell count as follows:

$$\text{MCV} = \frac{\textit{Haematocrit (l/l)}}{\textit{Number of erythrocytes (106/µl)}}$$

$$\text{MCH (pg)} = \frac{\textit{Haemoglobin g/l}}{\textit{Number of erythrocyte (106/ul)}}$$

$$\text{MCHC(g/dl)} = \frac{\textit{Haemoglobin concentration (g/dl}}{\textit{Haematocrit (l/l)}}$$

(Clark and Kruse, 1990)

Low MCV indicates microcytic (small average RBC size), normal MCV indicates normocytic (normal average RBC size), and high MCV indicates macrocytic (large average RBC size). Its normal range is 80-96 fL/red cells in adults (Clark and Kruse, 1990).

The study done in Addis Ababa, Ethiopia, demonstrated that haemoglobin, haematocrit and RBC counts are significantly associated with clustered components of metabolic syndrome in working adults (Nebeck *et al*., 2012).

The red cell distribution width (RDW) is the component of haematological parameters that quantifies variations in the size of circulating erythrocytes. The normal value for RDW is 13 ± 1.5%. it is calculated from the red cell volume distribution histogram in which the standard deviation of erythrocyte volume distribution is divided by MCV and finally the result is multiplied by 100 (Evans and Jehle, 1991).

$$RDW = \frac{SD\ of\ red\ cell\ volume}{MCV} * 100$$ (Evans and Jehle, 1991).

With higher values RDW reflects greater heterogeneity in cell sizes. It is advanced biomarker for multiple physiological impairments related to atherosclerosis and cardiovascular diseases (CVD) and is associated with increased risk of mortality and CVD events in patients with established coronary artery diseases (CAD) (Felker *et al*., 2007; Tonelli *et al*., 2008; Su *et al.,* 2014; Li *et al.,* 2015; Hou *et al.,* 2017; Adam *et al.,* 2018)

Platelets are very variable in size, metabolism, and functional activity. Size of platelet indicated to have certain association with diseases. Largest platelet for instance indicated to be more reactive and produces a greater quantity of thrombogenic factors (Leader *et al.,* 2012). A group of derived platelet parameters obtained as a part of the automatic complete blood count that may have diagnostic and prognostic value in certain diseases are called platelet indices. These are plateletcrit (Volume occupied by platelets in the blood), the mean platelet volume (MPV) and platelet distribution width (PDW) (Budak *et al.,* 2016). The mean platelet volume (MPV is used to assess platelet activity which is correlated with platelet size). Higher MPV is associated with the presence of cardiovascular risk factors (hypertension, type 2 diabetes etc.), chest pain due to acute coronary syndrome, and adverse outcome after acute coronary syndrome (Buttarello *et al.,* 2008; Chu*et al.,* 2010; Leader *et al.,* 2012; Sansanayudh *et al.,* 2014) and also indicated as a surrogate marker for MetS in clinical settings. A measure of platelet size variability called platelet distribution width that depends on the process of platelet production, fragmentation of cytoplasm of megakaryocytes and proplatelet formation (Buttarello *et al.,* 2008). It is shown that MPV has reverse correlation with MetS in female subjects and describe that it is significantly increased in adult patients who have diabetes, impaired fasting glucose, obesity and hypertension (Aypak *et al*., 2014).

There is a direct relationship between MPV and PDW in healthy state. In case of MetS platelets count, platelet distribution width (PDW), and mean platelet volume (MPV) levels and platelets/lymphocyte ratio were shown to be significantly higher in all patients with MetS (Abdel-Moneim *et al*., 2019).

### 1.2.4 Framingham risk score

Framingham risk score is the first cardiovascular diseases risk assessment tool that was developed from the Framingham Heart Study in the 1970s. It can estimate a patient's 10-year risk for myocardial infarction and cardiac death based on the CVD risk factors. These are gender, age, total cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, and cigarette use and give classification as low risk (< 10%), intermediate risk (10–20%), and high risk (> 20%) which is calculated online (Devine and Taylor., 2009; De Ruijter *et a*l., 2009 ).

## 1.3 STATEMENT OF THE PROBLEM

Cardiovascular disease is the leading cause of morbidity and mortality worldwide with continuous upswing prevalence of epidemic proportions in both developed and developing nation (Balakumar *et al*., 2016. In 2017, it was the reason for 17.8 million deaths universally out of which around 80% of total CVD deaths ensue in low- and middle-income countries (Mensah *et al*., 2020).Fifty percent (50%) of all these cardiovascular deaths in 2017 was caused by IHD (ischaemic heart disease) (Yuyun *et al*., 2020). In 2019 CVD is accounted for1/3rd of all deaths worldwide (9.6 million and 8.9 million deaths among men and women respectively) among these deaths>6 million deaths were occurring between the ages of 30 and 70 years (Roth *et al*., 2020). In china in 2014 it was accounted for 44.60% and 42.51% of all deaths in rural and urban areas respectively (Liu *et al*., 2019).

One of the cardiovascular diseases indicated to have higher morbidity and mortality globally is atherosclerotic cardiovascular disease (ASCVD) which is accounted for most CVD diseases. It is shown to cause 31% death in 2015 globally (Kim *et al*., 2019; Arnett *et al*., 2019) and 33–40% of all-cause mortality in USA and Europe respectively (Berry *et al.*, 2012). Study done on Korea adult patients also showed that it had 9.825% and 10.11% prevalence in 2014 and 2015 respectively (Kim *et al*., and 2019). Among ASCVD subtypes IHD and stroke were reported as the world first and third cause of death in 2013 (Abubakar *et al*., 2013). Similarly, ischaemic heart disease is indicated to be a cause of higher mortality in south Asian in USA (Volgman *et al*., 2018)

CVD is highly pronounced in low and middle income countries. For instance, In sub Saharan Africa CVD was accounted for around 1million death in 2013 (Keates *et al*., 2017) and systematic review also indicated that in eastern Ethiopia and in the capital city, Addis Ababa estimated prevalence was 7.2% and 24 % respectively for CVD (Misganaw *et al*., 2014). Additionally it was also shown that the prevalence of CVD is higher in patients visited hospital than the general population by having prevalence of 8% and 2% prevalence respectively (Angaw *et al* .,2021)

With regard to ASCVD risk factors hypertension, the leading risk for cardiovascular disease, seemed to be the major health problem worldwide with a prevalence and control rate of 40.8 and 32.3 respectively (Shafi and Shafi, 2017) and likely to cause 4.5% of current global disease burden and is prevalent in many developing countries as well as the

developed world (WHO, 2003).The number of adults with hypertension in 2025 was predicted to increase by about 60% (Kearney *et al*., 2005). For instance the study done in Saudi Arabia on the prevalence of CVD risk factors HTN was the most prominent risk factor having prevalence rate of 11.1% while DM had 5.6% prevalence rate (Gutierrez *et al.*, 2018).

Hypertension is the most common disease in developing countries where the rates of treatment awareness and control are very low. Its prevalence has been increasing in the sub Saharan Africa that ranged from 14.7% to 69.9%. The median prevalence was 29% that increase with age, higher in the urban compared with the rural population and minimal difference between men and female (Addo *et al.*, 2007; Ataklte *et al*., 2015). According to various studies done in Ethiopia it is known that hypertension is epidemic in the Ethiopian community with a prevalence of 13.2 in southwest Ethiopia, 25.1% in Bahir Dar city 27.9% in north west Ethiopia and 28.3 in Gondar Ethiopian adults. With the sub group analysis it is higher in urban area than rural and urban combined and it is almost similar in males and females (Awoke *et al.*,2012; Gudina *et al*., 2013; Anteneh *et al.*, 2015; Abebe *et al*., 2015) While diabetes prevalence ranged from 2.0%–6.5% (Bishu *et al*., 2019).

Beside to the prevalence of CVD and its risk factor's we also tried to observe the trend of diagnosing CVD in TASH which is the number one and the only national referral hospital in Ethiopia. The physician uses mostly hsCRP. But it is not available in TASH. Therefore the sample has to be send to EPHI and result may be announced after one or two days. Also in between this process there may be loss of patient result and the result may also be affected by the method of transportation as well as storage of the sample. Since that we want to look for the easily available parameters like the haematological parameters.

As described in few studies on haematological parameters in atherosclerotic cardiovascular diseases or in risk factors alone without cardiovascular disease, disturbance of various haematological parameters counts were observed. But, study comparing haematological parameters between atherosclerotic cardiovascular diseases and risk factors alone without cardiovascular disease was lacking. Therefore, the present study had tried to assess the pattern of haematological parameters in people with atherosclerotic cardiovascular diseases or in cardiovascular disease risk factors alone.

## 1.4 SIGNIFICANCE OF THE STUDY

Several epidemiological and clinical studies stated various inflammatory marker, adhesion molecules, cytokines, fibrinogen C-reactive protein as biomarker in CVD. But most of these are very expensive and also not easily available in Ethiopia. Therefore, it was better to look for cheap or economical and frequently available biomarker like haematological parameter. Very few study identified some haematological parameters as risk predictor of CVD. However, the array of haematological parameters before and after the onset of ASCVD specifically has not been extensively studied. In the current study, we pursued to explore the pattern of the haematological parameters in the presence of ASCVD risk factors and ASVD itself and gave indication on important informative indicator of risk for ASCVD and the presence of ASCVD. And we also believe that it may give new insight for forthcoming researcher on this area.

## 2 .OBJECTIVES OF THE STUDY

### 2.1 General objectives

The main aim of this study was to assess the pattern of haematological parameters in peoples with already established atherosclerotic cardiovascular diseases and those with ASCVD risk alone and also to assess its correlation with hsCRP in people

### 2.2 Specific objective

1. To evaluate the pattern of hematological parameters in people with established atherosclerotic cardiovascular disease (ASCVD) and in people with risk alone
2. To assess hsCRP in people with ASCVD and in people with risk alone
3. To analyze the correlation of hematological parameters with hsCRP in people with established atherosclerotic cardiovascular disease (ASCVD) and ASCVD risk group
4. To evaluate pattern of hematological parameters in ASCVD sub types.

## 3. MATERIALS AND METHODS

### 3.1 Study population and area

The study was conducted at Tikur Anbessa Specialized Hospital (TASH), department of Biochemistry, in collaboration with internal medicine, renal clinic, diabetes, neurology and cardiac clinic, Addis Ababa University, Addis Ababa, Ethiopia. The source population of the study was all patients visiting/attending renal, diabetic, neurology and cardiac clinic at TASH and the study population were all volunteer patients attending these clinics.

### 3.2 Study design and period

Prospective comparative cross sectional study was conducted where; from *October 2019-March2020* was proposal development, sample collection and lab analysis period and from *March 2020-june 2021* data entry, analysis and writing period.

### 3.3 Inclusion and exclusion criteria

#### 3.3.1 Inclusion criteria

- ✓ Able to give informed consent (HTN and /or DM)
- ✓ Patients with no known haematological abnormality
- ✓ Patients without acute infection

### 3.3.2 Exclusion criteria

- ✓ Pregnant women
- ✓ Lactating women
- ✓ Severely sick patients
- ✓ Unable to give informed consent
- ✓ Patients with known haematological abnormality
- ✓ Patients with acute infection
- ✓ Embolic ischemic stroke
- ✓ Patients with known diabetic complication; retinopathy, nephropathy, neuropathy

### 3.5 Sample size and sampling techniques

Study population were selected by using convenient sampling technique and double population formula were used to calculate the sample size of study population since we have compared two groups( groups with ASCVD risk factors vs. group with established ASCVD) by taking the prevalence of hypertension (0.28) (Anteneh *et al.*, 2015) and CVD (0.08) (Angaw *et al* .,2021).

$$n = (Z\alpha/2+Z\beta)^2 * (p1(1-p1) + p2(1-p2)) / (p1-p2)^2$$ ..... (Hickey *et al*., 2018)

Where;

$Z_{\alpha/2}$- is the critical value of the Normal distribution at α/2 (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96),

$Z_{\beta}$- is the critical value of the Normal distribution at β (e.g. for a power of 90%, β is 0.1 and the critical value is 1.28) and

**p1 and $p_2$** - are the expected sample proportions of the two groups

$$n = \frac{(1.96+1.28)^2 * (0.28(1-0.28)+0.08(1-0.08)}{(0.28-0.08)^2}$$

n =72.22 ≈ 72, by adding 10% non-response rate it becomes 82. But, we increased it to 100 (50 participants in each group) to increase the power of the study.

### 3.6 Study variable

#### 3.6.1 Dependent variable

- Hematological parameters:- white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PLT), platelet distribution width (PDW),
- Highly sensitive C-reactive protein (CRP)

#### 3.6.2 Independent variable

- Socio-demographic characteristics; age, sex, height, weight, BMI, waist circumference, alcohol consumption, cigarette smoking, exercise, types of diet, family history.
- Clinical variables; Types of diabetes, hypertension, systolic pressure, diastolic pressure and types of atherosclerotic cardiovascular disease.
- Lipid profile: Total cholesterol, HDL cholesterol, LDL cholesterol triglycerides

### 3.7 Sample collection

Five (5) ml of fasting venous blood was collected from each participant and 2.5ml delivered to tubes containing EDTA and 2.5 ml delivered to serum separator tube following aseptic condition. As soon as the collection is completed it was placed in ice box and transported to the laboratory, and for serum sample after leaving for minimum of 30 minutes at room temperature to allow coagulation, the coagulated blood was centrifuged at 3500 rpm for 10 minutes to separate serum from clotted blood. Then serum sample will be collected into eppendorf tube and analyzed. Sample which wasn't assayed within 24 hours of collection was stored at -20oc. For whole blood sample it is placed in ice box and transported to EPHI for analysis.

### 3.8 Data collection

Socio-demographic characteristics and other related data were collected from the participants by principal investigator via face to face interview using Amharic version structured questionnaire, reviewing patient's card and direct measurement of height, weight and blood pressure.

### 3.9 Laboratory methods

#### 3.9.1 Determination of haematological parameters

To determine complete blood count, SYSMEX was used that provides accurate and precise results through high-quality technology. This method accurately count and size cells by using measurable charges in electric resistance produced by nonconductive particle suspended in electrolyte (O Neil *et al.*, 2001).

#### 3.9.2 Principles of haematological parameters determination

A suspension of blood cells passes through a small orifice simultaneously with an electric current. A small opening (aperture) between electrodes is the sensing zone through which suspended particles pass. In the sensing zone, each particle displaces its volume of electrolyte. Sysmex measures the displaced volume as a voltage pulse, the height of each pulse being proportional to the volume of the particle (Blood, 2007).

#### 3.9.3 hsCRP determination

hsCRP is determined by using Roche/Hitachi Cobas 6000 analyser.

**Test principle**

Particle enhanced *immunoturbidimetric assay.* Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined *turbidimetrically.*at the wavelength of 546nm.

#### 3.9.4 Determination of total, HDL, LDL cholesterol and triglycerides

Total, HDL cholesterol and triacylglycerol which are mainly done to diagnosis and monitor lipid related diseases were determined by using spectrophotometric method while LDL is calculate by using fried Wald's formula (LDL= TC-(HDL-TG/5) by principal investigator and then repeated by using Roche/Hitachi Cobas 6000 analyser which is fully automated method at EPHI laboratory by clinical chemistry lab personnel. It works based on principle of enzymatic colorimetric test.

##### 3.9.4.1 Principle of total cholesterol determination

Cholestrolester + $H_2O$ Cholesterol ester Cholesterol + Fatty Acids

Cholesterol + $O_2$ Cholesterol oxidase cholest-4-ennone + $H_2O_2$

$2H_2O_2$ + hydroxyl benzoate + 4AA Peroxidase red complex + $4H_2O$

The colour intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance at 512 nm.

**3.9.4.2 Principle of triglycerides determination**

Triglycerides + 3 $H_2O$ LPL glycerol + 3 RCOOH

Glycerol + ATP GK glycerol 3 phosphate + ADP
Mg2+

glycerol-3-phosphate + O $GPO_2$ dihydroxyacetone phosphate + $H_2O_2$

$H_2O_2$ + 4-aminophenazone + chlorophenol peroxidase (benzaquinone-monomino) - phenazone + $2H_2O$ + HCl

The colour intensity of the red dyestuff formed is directly proportional to the triglyceride concentration and can be measured photometrical at wavelength of 505nm.

**3.9.4.3 Principle of HDL cholesterol determination**

HDL cholesterol ester + $H_2O$ CHER HDL cholesterol + RCOOH

HDL cholesterol + $O_2$ CHOD cholestenone + $H_2O_2$

$2H_2O_2$ + 4-Aminoantipyrine + $H_2O$ POD coloured pigment + $5H_2O$

The colour intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrical at wavelength of 600nm.

**3.9.4.4 LDL cholesterol determination**

Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reactions to the lipoproteins other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL and chylomicron is not determined.

LDL-cholesterol esters + $H_2O$ detergent cholesterol esterase

LDL cholesterol + free fatty acids

LDL-cholesterol + $O_2$ cholesterol oxidase Δ4-cholestenone + $H_2O_2$.

2 $H_2O_2$ + 4-aminoantipyrine) + $H_2O$ + H + peroxidise red purple pigment + $5H_2O$

### 3.10 Data processing and analysis

All the statistical analysis was performed using statistical package for the social science (*SPSS) ver. 25*. The variables distribution was tested for normality by using *Kolmogorov test* and transformed if necessary. Simple descriptive statistics such as mean, standard deviations, median and IQR was calculated for *continuous* variables and percentages were calculated for categorical variables. To compare socio-demographic, anthropometric and clinical characteristics Chi- square test was used and to compare normally distributed quantitative data Independent t-test was used while Mann Whitney U test was used for non-normally distributed data. Pearson or spearman correlation tests were also used as required. Multinomial logistic regression was done. The output data was interpreted using tables and/or charts, P value<0.05 was considered statistically significant for all analysis.

### 3.11 Data quality control and management

- The socio –demographic data and related data was collected by the PI
- Measurement of blood pressure was done by using a sphygmomanometer by professional nurses
- All the sample taking procedure PI and analysis of sample was carried out by EPHI clinical chemistry and hematology lab personnel's.
- Tools that were used to measure and analyze the test are standardized and automated
- Data code entering, verifying, and cleaning were performed with great care.

### 3.12 Ethical Approval

Before starting data collection, ethical clearance letter was obtained from the department research and ethics review committee, department of Medical Biochemistry, college of Health Science, Addis Ababa University. The purpose of the study was briefly explained for the study participants and they were informed that their response will be treated with strict confidentiality. Samples and data had been collected after the study participants gave

consent. Confidentiality, anonymity, neutrality, accountability, and academic honesty were maintained throughout the study.

### 3.13 Operational definition

**ASCVD-** participants having confirmed ASCVD, diagnosis of ischemic heart disease or non-cardio embolic ischemic stroke

**Case group-** group with already established ASCVD

**Comparison group**- group having one or more ASCVD risk factors

**Hypertension**- medical history of hypertension.

**Diabetes**-medical history of diabetes or on diabetic treatment during study period

**Cigarette smoking**-participants who smoke cigarette currently

**Alcohol consumption**-participants who have alcohol consumption habit currently

**Normal mean value of haematological parameters in Ethiopian adults**

- ✓ **Normal WBC count** - WBC count with mean value (6.0±1.8) × $10^9$/litre for male and 6.1± 2.2 in female
- ✓ **Normal differential WBC COUNT**-;
  - Monocytes = 6.7±1.7(males), 6.0±2.0(females)
  - Granulocytes = 55.1±12.3(males),54.3±12.5(females)
  - Lymphocytes = 35.2±10.3(males), 36.4±11.3) (females)
- ✓ **Normal RBC count** = (5.1±0.4)× $10^{12}$/liter (males) and (4.5±0.4) × $10^{12}$/liter (females)
- ✓ **Normal adult haemoglobin level** =16.1±1.1 (male) and 14.3±1.2 (female) g/dl;
- ✓ **Normal haematocrit level** =48.3%±3.4 (male) and 42.0%±3.2(female);
- ✓ **Normal platelets count**= 207±62 × $10^9$/liter ( males) and 202 ± 67 (females)

## 4. RESULT

### 4.1 Socio-demographic, anthropometric and clinical characteristics.

This study enrolled a total of 100 participant (50 cases (74% female) (*table 1*) and only quarter (24%) of the cases are in the age category of above 55 while two third (66%) of the comparison group were in this age category having significant P value of 0.000.

In the case group (36%) and in the comparison group (54%) were in the body mass index category of>25kg/cm2 (p=0.003) and the mean waist circumference in the case group was 41.5±12.16while it is36.91±9.94 in the comparison group (table 1). None of the participant in both groups was smoker, 76% and 82% of the comparison group and the case group had the habit of doing exercise 4-5 days per week (t*able 1*). About 68% of comparison group and 60 % of case group had the habit of eating meat occasionally (p=0.037).

Out of 50 cases included in the study 76% had a history of coronary heart disease and 24% had ischemic stroke (*table 3*).Out of 50 comparison group 11(22%) were diabetic (2%type 1, 20% type 2), 21(42%) were hypertensive and 18(42%) were both hypertensive and diabetic (8(16%) type 1,10(20%) type 2) (table 3).The other socio-demographic, anthropometric and clinical characteristics of both group are summarized in *Table1*.

According to Framingham risk score classification (figure 5) 25(50%), 14(28%), 11(22%) of comparison group are in the lower, moderate, and high risk group respectively.

***Table 1*. Comparison of socio-demographic, anthropometric characteristics and clinical characteristics**

| Variables | | Comparison group (n=50) | Case(n=50) | p-value |
|---|---|---|---|---|
| Gender | Female | 20(40%) | 13(26%) | 0.137 |
| | Male | 30(60%) | 37(74%) | |
| Age(yr.) | 15-35 | 1(2%) | 20(40%) | 0.000 |
| | 36-55 | 16(32%) | 18(36%) | |
| | >=56 | 33(66%) | 12(24%) | |
| BMI(kg/cm$^2$) | <18.5 | 0(0%) | 11(22) | 0.003 |

| | | | | |
|---|---|---|---|---|
| | 18.6-24.9 | 23(46%) | 21(42) | |
| | >25 | 27(54%) | 18(36) | |
| WC(cm0 | | 41.5±12.1 | 36.91±9.94 | 0.241 |
| Exercise | Occasionally | 5(10%) | 1(2%) | 0.739 |
| | 2-3 days/week | 4(8%) | 4(8%) | |
| | 4-5 days/week | 38(76%) | 41(82%) | |
| | Never | 3(6%) | 4(8%) | |
| Red meat eating | Occasionally | 30(60%) | 34(68%) | 0.037 |
| | 1days/week | 3(6%) | 7(14%) | |
| | 2-5 days/week | 4(8%) | 6(12%) | |
| | Never | 13(26%) | 3(6%) | |
| Vegetable eating | Occasionally | 7(14%) | 4(8%) | |
| | Days/week | 4(8%) | 4(8%) | |
| | 45days/week | 37(74%) | 41(82%) | 0.716 |
| | Never | 2(4%) | 1(2%) | |
| Fruit eating | occasionally | 21(42%) | 20(40%) | 0.929 |
| | Days / week | 7(14%) | 7(14%) | |
| | 4-5days/ week | 20(40%) | 22(44%) | |
| | Never | 1(2%) | 2(4%) | |
| Type of oil | Palm oil | 31(62%) | 36(72%) | 0.202 |
| | Sun flower | 6(12%) | 9(18%) | |
| | Non particular | 12(24%) | 4(8%) | |
| | Don’t know | 1(2%) | 1(2%) | |
| Cigarette smoking | Never | 50(100%) | 50(100%) | |
| Alcohol consumption | occasionally | 31(62%) | 36(72%) | 0.554 |
| | 1 bottle/day | 18(36%) | 13(26%) | |
| | Never | 1(2%) | 1(2%) | |

| | | | |
|---|---|---|---|
| DM | 12(24%) | 2(4%) | 0.000 |
| Type 1 | 11(22%) | 2(4%) | |
| Type 2 | 1(2%) | 0(0%) | |
| HTN | 20(40%) | 10(20%) | 0.000 |
| HTN and DM | 18(36%) | 8(16%) | 0.000 |
| Type 1 | 8(16%) | 0(0%) | |
| Type2 | 10(20%) | 8(16%) | 0.121 |
| SBP(mmHg) <120 | 14(28%) | 22(44%) | |
| 120-140 | 28(64%) | 25(50%) | |
| >140 | 8(16%) | 3(6%) | 0.128 |
| DBP (mmHg)<70 | 4(8%) | 6(12%) | |
| 70-80 | 36(64%) | 38(76%) | |
| >80 | 14(28%) | 6(12%) | 0.128 |
| ASCVD<br>CHD | -<br>- | 38(76%)<br>12(24%) | |
| Ischemic stroke | | | |

NOTE: P<0.05 is statistically significant, derived from Chi-square test. DM- diabetes mellitus, HTN-hypertension, SBP-systolic pressure, DBP-Diastolic Blood Pressure, ASCVD - atherosclerotic cardiovascular disease, CHD-coronary heart disease, WC-waist circumference.

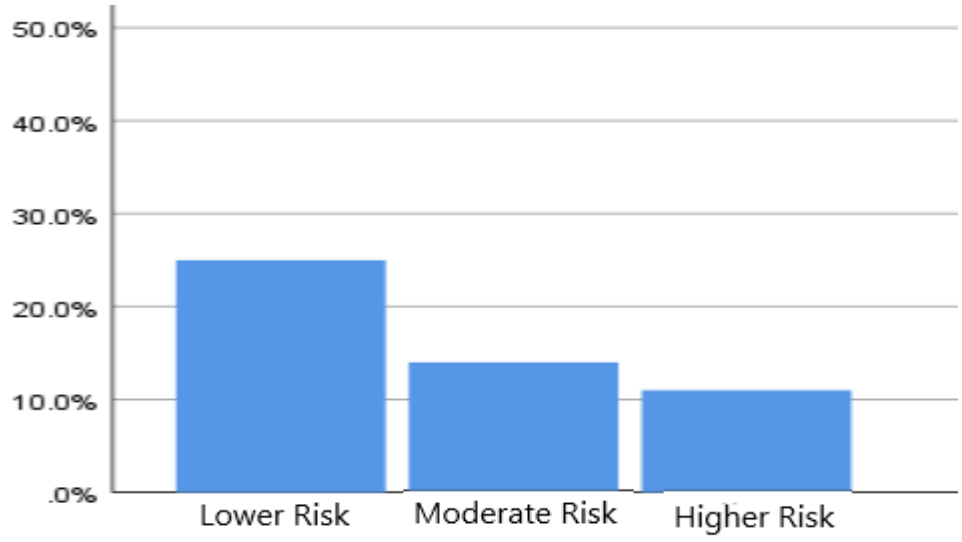


Figure 5 Classification of the comparison group according to Framingham risk score

**Table 2Comparison of anthropometric, demographic and clinical characterstics in ASCVD sub types**

| Variables | | CHD(38) | Ischemic stroke (n =12) | P-value |
|---|---|---|---|---|
| Gender | Male | 8(21.05%) | 6(50%) | |
| | Female | 30(78.9%) | 6(50% | |
| Age (year) | 13-35 | 18(47%) | 1(2%) | 0.011 |
| | 36-55 | 12(32%) | 6(50%) | |
| | >56 | 8(21%) | 5(41.6%) | |
| BMI (kg/m$^2$) | <18.5 | 11(28.9%) | - | 0.005 |
| | 18.6-24.9 | 16 (42.1%) | 4(10.5%) | |
| | >25 | 11(28.9%) | 8(21.05%) | |
| WC(cm) | | 38.67±3.27 | 36.41±11.33 | 0.287 |
| Systolic blood Pressure | <120 | 21(55.26) | - | 0.013 |
| | 120-140 | 13(34.2%) | 12(31.5%) | |
| | >140 | 4(1.05%) | - | |
| Diastolic blood Pressure | <70 | 4(1.05%) | 2(1%) | 1.000 |
| | 70-80 | 29(76.3%) | 8(6.6%) | |
| | >80 | 5(13.1) | 2(1%) | |
| Hypertension | | 13(35.1%) | 5(13.1%) | |
| Diabetes | | 10(26.3%) | 2(5.26%) | |

Analysis was done by using chi-square test. P<0.05 is statistically significant. BMI-body mass index, WC-waist circumference, CHD- coronary heart disease.

## 4. 2 Comparison of clinical laboratory results

### 4.2.1. Hematological parameters

When the hematological parameters of the two groups (case and comparison) compared (*table 3*) *mean± SD* of MCHC (33.32±1.01 vs. 33.7+0.68, p=0.031) and MPV (8.44+1.03 vs.9.32+1.16, p=0.00) were lower and MD (IQR) of MCV (89.52(5.50) vs. 87.5(4.25), p=0.006) and MONO (7.85(2.77) vs. 6.25(2.70), p=0.00) were higher in case group. While value of RBC, HCT, MCHC, HGB, NEUT, Lymphocyte, were lower non -significantly. But, WBC, MCH, RDW and PLT count were higher in the case group non -significantly.

Additionally, neutrophil lymphocyte ratio, platelet lymphocyte ratio were compared and shown to be higher insignificantly in the case group than in those comparison group (table 3 ).

**Table 3comparison haematological parameters in comparison and case group**

| Hematological parameter | Comparison group | | Case group | | |
|---|---|---|---|---|---|
| | Mean ± SD | Median (IQR) | Mean ±SD | Median (IQR) | P-value |
| Hgb *(g/dl)* | 15.23±1.47 | | 14.94±2.22 | | 0.436 |
| Hct*(%)* | 45.19±1.47 | | 44.77±6.63 | | 0.707 |
| RBC*(10^6/ul)* | 5.16±0.53 | | 5.03±0.67 | | 0.275 |
| WBC*10^3/ul)* | 6.41±2.75 | | 7.17±2.42 | | 0.086 |
| MCHC*(g/dl)* | 33.70±0.68 | | 33.32±1.01 | | 0.031 |
| RDW *(%)* | 13.69±0.95 | | 14.15±1.71 | | 0.106 |
| PLT(*10^3/ul)* | 245±67.60 | | 268±68 | | 0.142 |
| MPV*(fl)* | 9.32±1.16 | | 8.44±1.03 | | 0.00 |
| Neutrophil *(%)* | 54.31±10.45 | | 53.48±12.97 | | 0.726 |
| Lymphocyte*(%)* | 33.74±9.69 | | 33.14±11.36 | | 0.776 |
| MCV*(fl)* | _ | 87.5(4.25) | | 89.52(5.50) | 0.006 |
| Monocyte*(%)* | _ | 6.25(2.70) | | 7.85(2.77) | 0.00 |
| Eosinophil*(%)* | _ | 2.75(2.97) | | 2.35(4.78) | 0.926 |
| MCH*(pg)* | _ | 29.65(1.80) | | 29.95(1.77) | 0.155 |
| Basophil*(%)* | _ | 0.80(0.70) | | 0.65(0.50) | 0.426 |
| NLR(%) | 1.82± 0.88 | | 2.05 ±1.52 | | 0.350 |
| PLR(%) | 7.97±3.53 | | | 9.53± 5.98 | 0.117 |

Note: for parameters expressed in mean ± SD p value is derived from independent T test while it is derived from Mann Whitney U test for parameters expressed in median (interquartile range); p-value at 0.05 is significant.

The types of ASCVD (ischemic stroke and coronary heart disease) also compared (*Table -4)* with respect to hematological parameters and found that the mean ± SD of total WBC count (5.98±2.05vs7.55±2.43, p=0.048) and neutrophil count (46.4±9.36 vs. 55.72±13.23, p=0.028) were higher in the group with coronary heart disease (CHD) while lymphocyte was lower 40.03±8.78vs.30.96±11.30, p=0.024. Whereas,

the RBC count, HCT, MCHC, MCH, Hgb, RDW,PLT, P/L, N/L, BASO, EOS count didn't show significant difference between the two group.

**Table 4 Comparison of haematological parameters in ASCVD sub types**

| Variables | Ischemic stroke(n=12) | | Coronary heart disease(n=38) | | P-value |
|---|---|---|---|---|---|
| | Mean± SD | Median (IQR) | Mean ±SD | Median (IQR) | |
| WBC(10^3/ul) | 5.98±2.05 | | 7.55±2.43 | | 0.048 |
| RBC(10^6/ul) | 5.3±0.45 | | 4.97±0.72 | | 0.243 |
| Hgb (g/dl) | 15.27±2.89 | | 14.83±1.99 | | 0.552 |
| HCT (%) | 45.45±7.48 | | 44.55±6.43 | | 0.685 |
| MCHC(g/dl) | 33.39±1.63 | | 33.30±0.96 | | 0.800 |
| RDW (%) | 14.30±2.85 | | 14.09±1.20 | | 0.711 |
| PLT(10^3/ul) | 271.92±68.95 | | 267.39±91.07 | | 0.875 |
| MPV(fl) | 8.59 ±1.26 | | 8.39± 0.96 | | 0.570 |
| NEU (%) | 46.4±9.36 | | 55.72±13.23 | | 0.028 |
| LYM (%) | 40.03±8.78 | | 30.96±11.30 | | 0.024 |
| MCV(fl) | | 88.75(6.77) | | 90.35(5.47) | 0.369 |
| MONO (%) | | 7.65(3.10) | | 7.85(2.80) | 0.682 |
| EOS (%) | | 2.90(2.72) | | 2.35(3.16) | 0.601 |
| MCH(pg) | | 29.95(2.52) | | 29.95(1.63) | 0.927 |
| BASO (%) | | 0.75(1.80) | | 0.6(0.43) | 0.946 |
| N/L | 1.24±0.44 | | | 2.34±1.67 | 0.001 |
| P/L | 7.01±1.08 | | | 10.44±6.67 | 0.007 |

Note: for parameters expressed in mean ± SD p value is derived from independent T test while it is derived from Mann Whitney test for parameters expressed in median (interquartile range**)**

### 4.2.2 hsCRP

Highly sensitive C-reactive Protein(hsCRP) is significantly higher in the comparison group or in the group without established ASCVD but having risk factors for ASCVD (figure 6) significantly (2.38(2.98) vs. 0.95(1.98), P=0.005).

Logistic regression (Table 7) done using the comparison group as reference group, hsCRP was significantly associated (B=.437, 95%CI.1988-.966), P=.041) with the presence of ASVD risk factors.

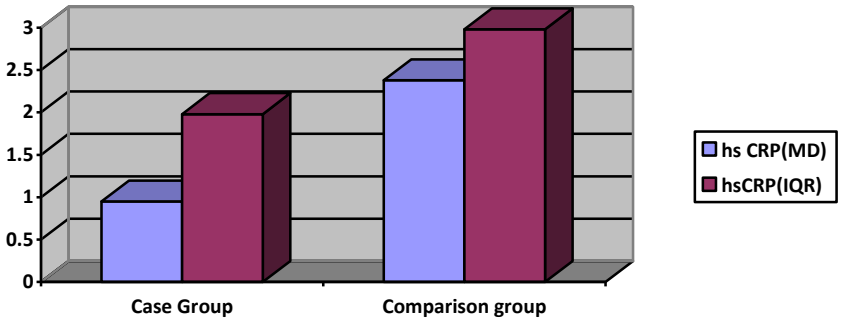


Figure 6 Comparison of hsCRP

Note: hsCRP- highly sensitive C-reactive protein, MD-median, IQR-interquartile range

### 4.2.3. Lipid profile

Lipid profiles (TC, TG, and LDL) values were non-significantly higher in the case group (table 5) than the comparison group. In the case group 8**%,** 18%, 22%, 20% and in the comparison group 12%, 16%, 34%, 32% had below the cut off value of TC, TG, LDL, HDL respectively (figure-7).

**Table 5 COMPARISON LIPID PROFILE**

| Lipid profile | Comparison group MD(IQR) | Case group MD(IQR) | P-value |
|---|---|---|---|
| TC(mg/dl) | 137.70(50.45) | 146.73(50.01) | 0.436 |
| HDL(mg/dl) | 35.45(8.33) | 36(9.90) | 0.495 |
| LDL(mg/dl) | 70.75(38.61) | 82(61.44) | 0.480 |
| TG(mg/dl) | 145.5(75.73) | 157.5(78.25) | 0.083 |

Note: P value is derived from Mann Whitney U test that is significant at P< 0.05. TC-total cholesterol, HDL-High density lipoprotein, LDL-low density lipoprotein, TG-triglyceride, MD-median, IQR-interquartile range.

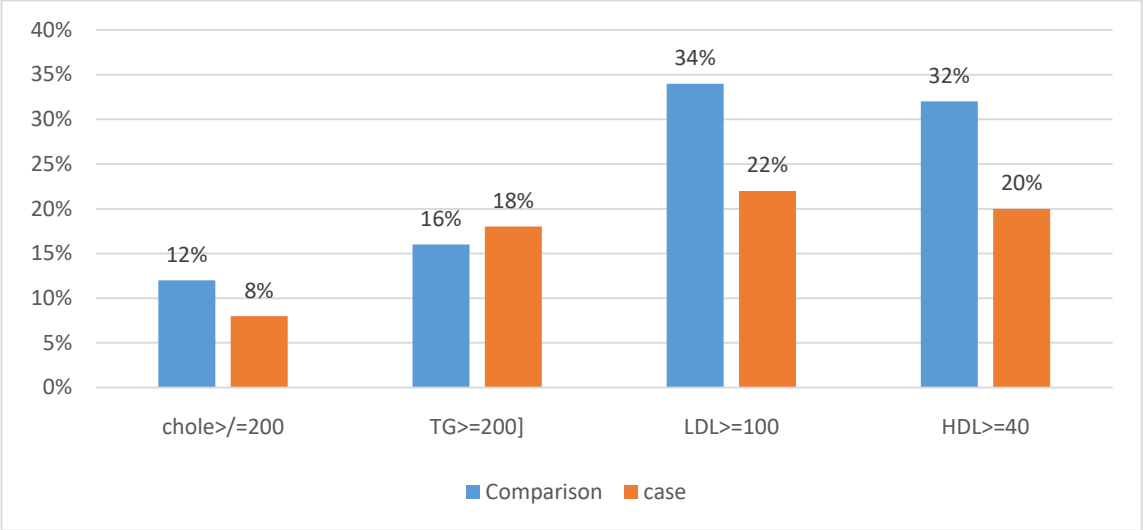


Figure 7. Percentage of participants having lipid profile above the cut off of point

Chole- cholesterol, TG- triglyceride, LDL-low density lipoprotein, HDL- high density lipoprotein

### 4.3. Correlation of hematological parameters with hsCRP

While we analyze correlation of hematological parameters with hsCRP (table 6) and only MPV (0.341(0.016)) (figure 6) had significant positive association in the comparison group. But, the rest of the hematological parameters had no any significant association.

Additionally, correlation of NLR, PLR with hsCRP (table 6) was also done and significance wasn't spotted.

**Table 6 Correlation of haematological profiles with hsCRP**

| Variables | Comparison group | Case |
|---|---|---|
| WBC(10^3/ul) | 0.025(0.867) | 0.176(0.125) |
| RBC(10^6/ul) | 0.122(0.403) | 0.221(0.128) |
| Hgb(g/dl) | 0.121(0.406) | 0.162(0.265) |
| Hct (%) | 0.223(0.123) | 0.183(0.194) |
| MCV(fl) | 0.132(0.367) | -0.006(0.969) |
| MCH (pg) | 0.007(0.962) | -0.062(0.673) |
| MCHC (g/dl) | -0.218(0.128) | -0.133(0.364) |
| RDW (%) | 0.241(0.092) | -0.017(0.906) |
| PLT(10^3/ul) | 0.143(0.326) | -0.258(0.073) |

| | | |
|---|---|---|
| Neutrophil (%) | -0.097(0.506) | 0.221(0.124 |
| Lymphocyte (%) | -0.019(0.896) | -0.237(0.101) |
| Monocyte (%) | 0.163(0.264) | 0.020(0.893) |
| Basophil (%) | 0.135(0.351) | -0.198(0.172) |
| Eosinophil (%) | 0.236(0.102) | -0.134(0.360) |
| Neutrophil lymphocyte ratio | -0.002(0.992) | 0.195(0.174) |
| Platelet lymphocyte ratio | 0.187(0.193) | -0.004(0.978) |

P<0.05(2-tailed), derived from *Pearson and spearman rank correlation*

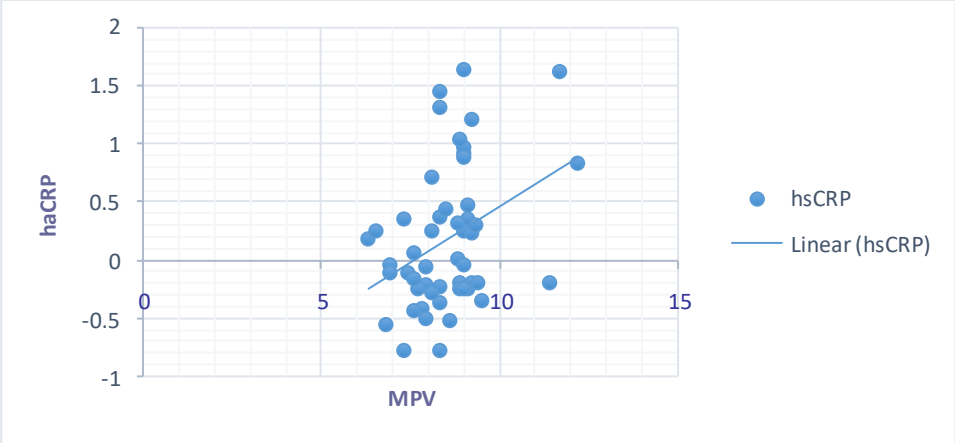


Figure 8 Correlation of hsCRP and MPV. In case group,p=0.016, r =0.341, derived from Pearson correlation test. hsCRP- highly sensitive C-reactive protein, MPV-mean platelet volume

## 4.4. Prognostic value of haematological parameter

All the hematological parameters related to the CVD and its risk with significant <0.05 and nearly close to significances were used in multinomial logistic regression(Table 7), WBC, MCV, RDW and MONO are significantly associated with pre-existing ASCVD while MPV significantly associated with the presence of the ASCVD risk. Additionally, hsCRP is significantly associated (Odds ratio (B) =.437, 95% CI 0.1988-0.966), P=.041) with the presence of ASCVD risk factors.

**Table 7 Predictive value haematological parameters**

| Variables | Regression coefficient (B) | Adjusted Odd ratio (B) | P-value | 95% CI (confidence interval) | |
|---|---|---|---|---|---|
| | | | | Upper bound | Lower bound |
| WBC | 0.281 | 0.755 | 0.033 | 0.583 | 0.977 |
| MONO | 0.500 | 0.607 | 0.000 | 0.463 | 0.795 |
| RDW | 0.231 | 1.512 | 0.034 | 1.037 | 2.203 |
| MPV | -0.828 | 2.289 | 0.008 | 1.245 | 4.206 |
| MCV | 0.122 | 1.135 | 0.013 | 1.029 | 1.252 |
| hsCRP | -0.828 | 0.437 | 0.041 | 0.198 | 0.966 |

Reference category comparison group. P<0.05 WBC- white blood cells, MONO-monocyte, RDW-red cell distribution, MPV-mean platelet volume,-MCV- mean cell volume, hsCRP-highly sensitive C-reactive protein

## 5. DISCUSSION

This study had focused on the role of complete blood count in the presence (case group) and absence of established ASCVD (comparison Group) which was the first study to be done in Ethiopia. We had tried to discuss this by referring various related studies as follows.

In present study there was association of total leukocyte count with the pre-existing ASCVD which is similar with previous study done by kim *et al* and Waterhouse *et al* in Korea and Ireland respectively (waterhouse *et al*., 2008; Kim *et al*., 2017) and also different investigator had showed that there is higher total leukocyte count in CVD diseased groups than the non-diseased one (Yan *et al*., 2020; Madjid*et al.*, 2004; Drague*t al*., 2008). There are also additional supportive studies like study conducted by Lasek-Bal and his colleagues that showed positive association of high leukocyte with a worse prognosis regarding the course of acute stroke and WBC was higher in group with symptomatic atherosclerosis than in patients with no clinical features of atherosclerosis (Lasek-Bal, *et al*., 2019). Studies by Lee*et al* also revealed that higher WBC is associated with increased incidence of the two prominent ASCVD types (ischemic stroke and CHD) (Lee *et al.*, 2018).

With regard to the WBC sub types this study revealed that it was only the monocyte which is associated with pre-existing ASCVD. This is in line with various studies (Swirski *et al*., 2007; Weber *et al*., 2008; Woollard and Geissmann, 2010) and also some review article confirmed this by analyzing various studies (Potteaux *et al*., 2007; Galkina, and Ley, 2009; Rahman, and Fisher, 2018). This would be due to the nature of monocyte to be involved in both innate and adaptive immunity and it has high trafficking and plasticity ability, it is highly recruited to the site of inflammation and differentiates in to lipid phagocytizing cell, macrophages. These macrophages aggravate the formation of atherosclerotic lesion by secreting metalloproteinase enzyme and ROS in atherosclerotic lesion (Fayad *et al*., 2018).

On the other hand study by waterhouse *et al* had revealed that monocyte is associated with CVD risk (waterhouse *et al*., 2008). And also some studies found out the role of other WBC subtype in relation to CVD. For instance, study by fan *et al* showed that higher NLR and decreased eosinophil is associated with ischemic stroke mortality rather than monocyte (fan *et al*., 2018). Verdoia and his colleagues also showed that NLR is independently associated with the prevalence and severity of coronary artery disease (CAD) (Verdoia *et al.*, 2016).

In this study higher RDW associated with the ASCVD morbidity rather than with the risk which is consistent with various studies like Lappé *et al* study that was done on 1,489 patients with CAD and a population of 449 normal (No-CAD) in which RDW was associated with mortality in patients with stable angina pectoris or normal coronary subjects (Lappé *et al*., 2011). Similarly, other studies had also reported that elevated RDW was associated with mortality in patients with stable angina pectoris and MI respectively (Uyarel *et al*., 2012; Sangoi *et al*., 2014). Additionally, study done in United States on adults by Ani, and Ovbiagele also revealed that the mean RDW was expressively higher among persons with known a stroke in comparison to group without a stroke (Ani, and Ovbiagele, 2009). The explanation for this may be due to the presence of inflammation pro-inflammatory cytokines are produced or released continuously. Those cytokines can interact with erythropoietin in the bone marrow and results in the reduction of RBC synthesis and also result in elevation in the number of immature RBC by suppressing RBC maturation which is detected by higher RDW levels (Uyarel *et al*., 2012; Lassale *et al*., 2018).

On the other hand, the epidemiological study done in Taiwan reported that elevated RDW is associated with increased risk rather than with development of the disease (Chen *et al*., 2010).

In our study MCV was significantly higher in the case group than the comparison group. Similarly, study done by Solak *et al* showed MCV as a predictor of composite CV events independent of major confounding factors and it is associated with endothelial dysfunction (Solak *et al*., 2013). Consistently, Myojo and his colleagues also showed that higher MCV is significantly associated with all-cause and cardiac mortality (Myojo *et al*., 2012). In line with this Tulubas *et al* who investigated ‘’MCV and MCH values in coronary artery patients with positive Gensini score’’ also observed that angiographic positive CAD group has higher MCV than angiographic negative CAD group significantly (Tulubas *et al*., 2013).

MPV is one of the platelet indices which estimate the size of individual platelet and it is higher expressively in the comparison group as compared to cases. Many studies done on role of MPV in various inflammatory disease revealed that it has inverse correlation with the level of inflammation (Douda *et al.,* 2006; Gunluoglu *et al*., 2014; Safak *et al*., 2014; Zareifar *et al*., 2014). Similarly, the study of Ihara *et al*. Showed that MPV is lower in angiographic positive group than angiographic negative group (Ihara *et al*., 2006). The longitudinal study done in Brazil on the association of platelet volume with the Framingham

risk score pointed out that elevated MPV is significantly related with Framingham risk. That means it increases as the Framingham risk score increase (Maluf *et al*., 2016). This controverts with many studies; for instance, High MPV indicated to be correlated with the severity of the coronary atherosclerosis in the study done by Murat *et al* on 395 patients with ACS (Murat *et al*., 2013). Similarly in the study conducted by Henning et al on a total of 518 patients conveyed that high MPV is associated with coronary heart disease (Henning *et al*., 2002).

The lower MPV in case group might be due to use of antiplatelet drugs while the comparison groups might not be taking it. But, we didn't investigate the treatment status of our study participants and it could be new insight for the future studies.

Highly sensitive C-reactive protein (hsCRP) had significant association with the presence of the risk in comparison group and higher significantly in these groups as compared to cases in current study. The decrement in the presence of the disease also seen in the cohort study done by the Taheri and his colleagues in which level of hs-CRP is lower in patients with CVD than without CVD (Taheri, *et al*., 2017).Some studies revealed that determination of hsCRP is useful in individuals with inter-mediate cardiovascular risk (10---20% risk at 10 years) in order to improve risk stratification and clinical management (Greenland *et al*., 2010: Silva *et al*., 2012;). This is also confirmed by the result of Seo, *et al* study in which The levels of hsCRP significantly increased from the low to the very high risk group (Seo *et al*., 2013) and also shown to have significant association with the CVDs like CHD, MI, stroke risk (Ridker *et al*., 2000; Ridker *et al*., 2002; Ridker *et al*., 2003). In reverse, cross sectional study done by Chowta and his colleagues revealed that there was higher mean hsCRP level in patients with CVD than those without CVD (Chowta *et al*., 2012).

The lower hsCRP in the already established ASCVD group may be due to that peoples in this group might have the chance to take drugs like statin than the comparison group that can affect or reduce CRP level or it may be due to that they would have started to change their life style by knowing that they developed ASCVD. Unfortunately, we didn't go through the type of drugs that the study participants were taking and this might require further investigation.

Among the hematological parameters it is only MPV that showed significant positive association with hsCRP in group with the already established ASCVD. This result was in

concordant with study that conducted on 84 IHD patients and found that there was significant correlation between MPV and hs-CRP levels (Yarlioglues *et al*., 2011). Which was also supported by Kaya *et al* study conducted in 2010 on the relationship between MPV and hsCRP in dipper and non-dipper hypertensive patients and revealed that they have significant positive association in the non-dipper hypertension which had around three times risk of atherosclerotic events (Kaya *et al*., 2010). This relationship was also demonstrated in the study piloted by Yazici *et al* and Yarlioglues *et al* on platelet indices in rheumatoid arthritis and relationship between mean platelet volume levels and subclinical target organ damage in newly diagnosed hypertensive patients respectively (Yazici *et al*., 2010; Yarlioglues *et al*., 2011).

There are many studies in controversy to this; in 2001, Kapsoritakis and his colleagues conveyed that there is a significant inverse relationship between MPV and hsCRP (Kapsoritakis *et al.,* 2001) and a cross sectional study done on 100 children with diagnosis of infectious and inflammatory disease in 2014 revealed that MPV and CRP have negative relation (Zareifar *et al;* 2014).

According to analysis of hematological parameter in ASCVD sub types higher WBC is observed in coronary heart disease than ischemic stroke. But there is no ample evidence from other studies to discuss this. Many studies that have done on ischemic and coronary heart disease separately had showed there is elevated leukocyte in the presence of coronary heart disease as well as ischemic stroke (Madjid *et al*., 2004; Haim *et al*., 2004; Koren-Morag *et al*., 2005; Lee *et al*., 2018) and why and how WBC is higher in CHD than ischemic stroke is not explained yet and we recommend the forthcoming researchers to investigate and proof it.

With regard to WBC sub type even though it is in the normal range neutrophil, lymphocyte, *NLR* and *PLR* was higher in CHD this may be due to the presence of high number of participants as well as high number of participants with diabetes, hypertension and other clinical variables in group with CHD than ischemic stroke, which may result in higher inflammation and stress (Sutherland *et al*., 2004). This could also be an explanation for the above result. But, it needs farther investigation

With respect to lipid profile, total cholesterol was higher in case group which was similar with previous study that showed positive association between TC and CVD mortality, largely due to IHD (Kwon *et al*., 2019). Compelling this study by Jeong and his colleagues reported

that higher total cholesterol has positive association with CVD incidences (Jeong *et al*., 2018). Faergeman and his colleagues also reported that increased TC level is associated with high risk of CVD events in statin treated patients (Faergeman *et al*., 2008). In reverse to this study done on Sudan patients showed that TC is lower in peoples with CHD than controls (Musa *et al*.,2013) which is also similar with Japanese study (Nago *et al*., 2011) and cohort study done on Korean (Bae *et al*., 2012).

According to current study triglyceride was higher in case group similar to study that showed high level TG is associated with CHD (Miller *et al*. 2008). Correspondingly indicated that higher TG is associated with higher CVD mortality (Mazza *et al*., 2005).Which is also consistent with various previous studies (Assmann, 2001; He *et al*., 2004).

LDL cholesterol, the prominent lipoprotein in atherosclerosis was also higher in case group than the comparison group, which is consistent with various previous studies. For instance study by Hedayatnia and his colleagues indicated that higher LDL is associated with total CVD events (Hedayatnia *et al*., 2020). Similarly, study on European whites also showed there is positive association between LDL level and CVD incidence (Wallace *et al.*, 2008)

In this study we observed that there was no detectable difference in the value of HDL level between the two groups but when we saw roughly it seems it is higher in the case group. In many studies it was shown that HDL level has inverse relation with cardiovascular events (Barter *et al*., 2007). In contrast to this some genetic and pharmacological studies as well as some literature reviews indicated and suggested that it is difficult to predict CVD event or risk by measuring HDL cholesterol concentration and it may not show any quantity disturbance or it may even elevated in the presence CVD and they suggested that it is better to focus on HDL functional quality than quantity (Navab *et al*., 2011; Rader and Hovingh, 2014; Vitali *et al*., 2017; Nicholls and Nelson, 2019).

As it has been explained TG, TC, and LDL are the main etiology for the ASCVDs and their elevation in the case group than the comparison group was expectable results (Hlaing and Park, 2013). But, for HDL it needs more investigation.

Generally, in addition to inflammation and other clinical factors, the presence of different age distribution, BMI and life styles like eating habit, drinking habit, exercising could also results in the presence of different pattern of haematological and biochemical parameters between the comparison group and the case group (Schaan *et al*., 2007; Shamai *et al*., 2011; Ebrahimi

*et a*l., 2016; Yen Jean *et al*., 2019; Singh *et al*., 2021; Feriel *et al* 2021). But it also needs further explanation by using more comprehensive study.

## 6. CONCLUSION AND RECOMMENDATION

This prospective comparative cross sectional study was aimed to compare the pattern of hematological parameter in group with confirmed ASCVD vs. the risked group. WBC, monocyte, RDW and MCV was associated with ASCVD morbidity while MPV and hsCRP had association with the presence ASCVD risk factors. And also MPV and hsCRP had significant positive correlation in the group with ASCVD risks. Therefore, using this affordable routinely tested and easily available tests might help to identify future ASCVD risk as well as the current ASCVD morbidity.

The current study also showed new research direction by revealing that hsCRP and some CBC parameters was higher in comparison group than the group with already established ASCVD which is different from pre-existing proofs and needs further intensive investigation.

## 7 Strength and limitation

This study has strength by being the first study to give a new insight on the pattern of hematological parameters in pre-existed ASCVD and ASCVD risk factors and it also gives new information on the existing proofs. Besides this it has various limitation; sample collection and measurement was a single time measurement it didn't record changes over time. The numbers of participants were small and also the type of medication that the participants taking may affect pattern of hematological parameters and could result in the occurrence of bias wasn't assessed. There may also be selection, misclassification,recall bias regarding the participants.

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## ANNEXES

### Annex 1 Study information sheet (English Version)

Research title:Cross sectional comparative study of hematological parameters in people with established atherosclerotic cardiovascular disease (ASCVD) and those with ASCVD risk alone**.**

**Investigator:** Ebsitu Abate

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**Introduction**

You are invited to take part in a research study. Before you decide, it is important for you to understand why the research is done and what it will involve. Please take a time to read or listen the following information carefully and discuss it with others if you wish.

**Purpose of the study**

The aim of this study is to compare hematological parameters in people with established cardiovascular disease and those with cardivascular risk alone**.**This will be important to implement available, less expensive diagnostic method, which will be important for better treatment strategies and improve prognosis of patients with vascular complication in the future.

**Procedure**

If you agree to participate in this study, we will require the following:

 We will ask you various questions related to the study.

**Potential risks and Discomforts**

There will be minor discomfort during blood sample collection. During sample collection, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

**Confidentiality**

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that

you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

**Sharing the result**

At the end of the study, the study findings will be published and they will be disseminated to other scientists through publication, and also to your doctor.

**The right to refuse**

The participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also refuse to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result for free.

**Annex 3. Consent Form (English Version)**

Patient code number _____

By name below, I confirm that I have read and understood this informed consent. I understand that this is a research study and that my participation is voluntary. I understand that I may change my mind about participating at any time, without my medical care or legal rights being affected. I have had the opportunity to ask questions and my questions have been answered. I have been given adequate explanation and understand the purpose, procedures, risks and benefits of the research study. By signing this form, I give my permission for the researchers to have access to my blood sample for the study.

| | | |
|---|---|---|
| Name of participant | date | signature |
| Name of investigator | date | signature |

Participant code no. _____

Participant phone no._____

**Demography, anthropometry, blood pressure and glucose level**

| Question | | Response |
|---|---|---|
| 1 | Gender | Male 1<br>Female 2 |
| 2 | Age | _____ yr |
| **Anthropometry** | | |
| | Weight (Kg)<br>Height(cm) | |
| | Body Mass Index (kg/cm2) | |
| | Waist circumference | In Centimetres (cm)<br>└──┴──┴──┘ . └──┘ |
| | Blood pressure | Systolic (MmHg)<br>Diastolic (MmHg) |
| **PHYSICAL ACTIVITY** | | |
| Do you perform physical exercise? | | YES<br>NO |
| How long a day | | □Occasionally □4days □3day<br>□2days □other_____ |

| **Dietary habits** | | |
|---|---|---|
| No | **Type of diet** | |
| 1 | Do you eat red meat? | Yes 1<br>No 2 |
| 2 | If 'yes' how often? | Occasionally **1**<br>1 day per week **2**<br>2-5 day per week **3**<br>> 5 days **4**<br>Don't know **5** |
| 3 | Do you eat vegetables, | Yes **1**<br>No **2** |
| 4 | If 'yes' how often? | Occasionally **1**<br>1 day per week **2**<br>2-5 day per week **3**<br>>5days **4**<br>don't know **5** |
| 5 | Do you eat fruits or? | Yes 1 No 2 |
| 6 | If 'yes' how often? | Occasionally **1**<br>1 day per week **2**<br>2-5 day per week **3**<br>> 5 days **4**<br>Don't know **5** |

| | | |
|---|---|---|
| 7 | What type of **oil or fat is most often** used for meal Preparation in your household? | Vegetable oil**1**<br>Palm oil **2**<br>coconut oil **3**<br>sunflower oil **4**<br>None in particular **5**<br>None used **6**<br>Don't know **7**<br>Others **8** |

| | | | |
|---|---|---|---|
| **BEHAVIOURAL MEASUREMENT** | | | |
| A) Tobacco use | | | |
| 1 | Do you smoke? | Yes 1<br>No 2 | |
| 3 | On average, **how many** of the following do you smoke each day? | 1 cigar daily 1<br>1 pack per day 2<br>□ other_____3 | |
| B) **Alcohol Consumption** | | | |
| 1. Do you consume alcohol<br>2. If yes how often**?** | | □YES □NO<br>□occasionally<br>□1 bottle alcohol daily<br>□2-5bottle alcohol daily<br>□other | |
| **Pathological information** | | | |
| | Hypertensive only | a. Yes 1<br>b. No **2** | |
| | Hypertensive with diabetes | a. YES b. NO | |
| | If 'yes' types of diabetes | a. Type 1 b. Type 2 | |
| | Nephropathy | a YES b NO | |
| | Neuropathy | a. YES b. NO | |
| | **Cardio vascular problem** | a YES b. NO | |
| | If yes which one | A)Ischemic stroke1<br>**a)** thrombotic<br>**B) Coronary artery identified with 2**<br>**a )** clinical diagnosis of stable angiography 1<br>**b)** History of myocardial 2 infarction<br>**c)**Echocardiography evidence 3 of coronary artery disease<br>**C)** Peripheral arterialism 3 identified with a Doppler of ultrasound of lower extremities artery<br>b) angiography<br>d) others | |

**Annex 4. Consent Form (Amharic version)**

የተሳታፊዎችስምምነትማረጋገጫ

የሚስጥር ቁጥር

**የተሳታፊዉ ስም**

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “የደም□N□ ጥበት ያለባቸዉ ሰዎችን የደም አይነቶች መጠንን የደም □N□ ጥበት ሳይኖርባቸዉ ለደም ጥበት የሚያጋልጡ ችግሮች ካለባቸው ሰዎች ጋር ማወዳደር እና ስአርፕ (CRP) ከተባለ ፕሮትን ጋር ያለዉን ግኑኝነት ማየት።” ጥናት ላይ በቂ ገለጻ ተደርጎልኛል። ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጾልኛል።የጥናቱንም አላማዎችም ተረድቻለሁ።

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል። በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል። ስለዚህ ለዚህ ጥናት መረጃናየስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነትነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ።የዚህ ጥናትተሳታፊ በመሆኔ የማገኘውጥቅም የሁሉን ምምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ። በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅየተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

ፊርማ _____ ቀን _____

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

**Annex 6. Study information sheet (Amharic version)**

አዲስአበባዩኒቨርሲቲ

የጤ ናሳይንስ ኮሌ ጅ

የሕ ክም ናፋካልቲ

ባዮኬሚ ስተሪ ት/ክፍል

የጥናቱ ተ ሳታ ፊዎ ች የመ ረጃ ቅፅ

የጥናቱ ባለቤት፡እብ ስቱ አባተ

አድራሻ፡- ሞ ባይ ል 0912632054

ኢ-ሜ ይል፡- ebsitu.abate@aau.edu.et

**መግቢያ፡**-እባክዎ በዚህ ጥናት ላይ ለመ ሳተፍ ከመ ወሰኖ በፊት ጥናቱ ለም ንእደሚ ካሄድና ም ንም ንዓይነት

ነገሮች እንደሚ ያስፈልጉት ለማ ወ ቅ ጥቂት ግዜ ይወስዱና የሚ ከተለውን ስለጥናቱ በተመ ለከተ መ ረጃ ይመ ልከቱ አስፈላጊ ከሆነም ከሌሎ ች ሰዎች ጋር ይወያዩ። ማ ንኛው ም ግል ፅ ያልሆነ ነገር ካለ ወይም ተጨ ማ ሪ መረጃ ከፈለጉ የጥናቱ ባለቤትን መጠየቅ ይችላሉ።

**የጥ ናቱ አላማ፡-**

የዝህ ጥናት አላማ *የደም* □*N*□ *ጥበት ያለባቸዉ* ሰዎች የደም አይነቶች መ ጠንን*የደም* □*N*□ *ጥበት* ሳይኖርባቸዉ ለደም □*N*□ ጥበት የሚ ያጋል & ችግሮች ካለባቸው ሰዎች ጋር ማ ወዳደር እና ስአርፕ (crp) ከተባለ ፕሮትን ጋርያለዉን ግኑኝነት በማ የት በዋጋ የተሻለውን እና በቀላሉ ተገኝቶ ለደም ጥ በት የመ ጋለጥ እድላችንን ለማ ወቅ የሚ ረዳንን የሚ ርመ ራ አይነት አገራችን ዉ ስጥ ተግባራዊ ማ ድረግ።

የጥናቱ ሂደት፡- በዚህ ጥ ናት ላይ ለመ ሳተፍ ፍቃ ደ ኛ ከሆ ኑከእርሶ የሚ ጠ በቁት የሚ ከተሉት ናቸው።

ም ርም ሩን ለመ ስራት የሚ ያስፈልገው የደም መ ጠ ን 5 ሲሲ ጥናቱን ለመ ስራት ብ ቻ እሚ ው ል።

የጥናቱ ባለቤት ጥናቱን በተመ ለከተ አንዳንድ ጥ ያቄ ዎች ን ሊጠ ይቅይችላል።

**ጉዳት፡**-ከዚህ ጥናት ጋር በተያያዘ በጤ ናም ሆ ነ በሚ ያገኙት ተገቢ ህክም ና ምንም አይነት ጉዳት ስለማያስከትል አይስጉ።

**ሚ ስ ጥ ራ ዊ ነት**፡-ማ ንኛውም ከዚህ ጥናት ጋር የተያያዘ የግል መ ረጃሚ ስ ጥራ ዊነት የተጠበቀ ነው ።

ስለዚህ የጥናቱ መረጃ ይፋ የሚ ሆነው ለእርሶ ብቻ ነው። ስለሚ ወ ሰደው ማ ንኛው ም መ ረ ጃ ዎ ች ሆነ የጥናት ው ጤ ት ለማ ሰራጨ ት በስም ሳይሆንበሚ ስጥር (ኮድ) የሚ መ ዘገብ ይሆናል።

**የተ ሳት ፎ መ ብ ት፡**-በዚህጥናትመ ሳተፍ ሙ ሉ በሙ ሉ በርሶፍቃ ድ የተመ ሰረተ መ ሆ ኑን ልናሳስብ

እንወዳለን። በመ ሆኑም በማ ንኛው ም ግዜ ም ንም ዓ ይነት ም ክንያት ሳይሰጡ ከጥናቱ ራስን የማ ግለል መ ብት የተጠበቀ ነው። የሰጡ ት ደም ናሙ ና ለዚህ ጥናት እንደሚ ው ል ማ ድረግ በእርሶ ሙ ሉ ፈቃድ ብቻ ሲሆንበጥናቱ ላይ ለመ ሳተፍ መ ወሰንወይም አለመ ወ ሰንመ ድሐ ኒት ወይም ሌላየጤ ና አገልግሎት የማ ግኘትመ ብትአሁንምሆነ ለወደፊቱ ምንምአይነት ተፅእኖ አያሳድርብዎትም።

ANNEX7 me‡Q

**U) የግል መ ረጃ**

1. የጥናቱ ተሳታፊ ሙ ሉ ስም፡ _____

2. የሚ ስጥርቁጥር፡ _____

3. መ ጠ ይቁ የተሞላበት ቀን፡ _____

4. መጠ ይቁ የተሞላበት ቦታ፡ _____

u) ìUb™Š# A³ Sn- UZB mré

wND 1 s_T 2

ADì…-

**ለ) የሰው ነት ልኬት**

ክብደት (ኪግ) _____ ቁመት (ሜ) _____

ywgB z&Þ¶_____s_ì…

**የደም ግ ፍት መ ጠ ን-**

**ሐ) የአካል ብቃት እንቅስቃሴ**

1. የአካል ብቃት እንቅስቃሴ ያደርጋሉ?

2. □ አዎ□ አይደለም

3. ለቀደመ ው ጥያቄ መ ልሶአዎ ከሆነ፡በሳም ንት ለም ንያህል ጊዜ?

□ አልፎአለፎ

□ 3ሰአት

□ 5ሰአት

□ 10ሰአት

□ ሌላ: _____

**መ) የአልኮል መ ጠ ጥ**

1. የአልኮል መ ጠ ጥ ይ ጠ ጣ ሉ?

□ አዎ□ አይደለም

2. ለቀደመ ው ጥያቄ መ ል ሶአዎ ከሆነ፡በቀንም ንያህል?

□ _በየቀኑ …….1

□ በሳምንትከ5-6 ቀን…….2

□ በሳምንትከ 1-4 ቀን…….3

□ በወርከ 1-3ቀን…….4

□ በወርከአንድግዜያነሰ……5

**ሠ) ማ ጨ ስ**

1. ያጨ ሳሉ?

□ አዎ□ አይደለም

4 አዎ ከሆነመ ልስዎ ምንያክል

□ 1 ስጋራ በቀን……1

□ 1ፓኬት ስጋራ በቀን…….2

□ ሌላ……3

**ረ) የአመ ጋ ገብ ሁኔታ**

1. ቀይ ሥ ጋ ወይም የታሸገ ስጋ ይጠ ቀማ ሉ

□ አዎ □ አልመ ገብም

2. አዎከሆነመ ልስዎ በሳም ንትለም ንያክልጊዜ?

□ አልፎአለፎ□ በሳምንት1 ቀን□ በሳም ንት 2-5 ቀን □ ሌላ: _____

3. አትክልት፣ፍራፍሬ ወይም ጥራጥሬዎችን ይመ ገባሉ?

□ አዎ□ አልመ ገብም

4. አዎ ከሆነመ ልስዎ በሳምንት ለም ንያክልጊዜ?

□ አልፎ አለፎ□ በሳምንት1 ቀን□ በሳም ንት 2-5 ቀን□ ሌላ:

6. ም ግብ ዎን ለማ ዘጋጀት ም ን አይነት ዘይት ይጠ ቀማ ሉ

yአTKLT zYT 1

yzN²² zYT 2

y××³T zYT 3

ys&F zYT 4

አlYM 5

aLeqMM 6

a®ˆQM 7

l_® 8

**ተ) የጤ ና ችግሮች መ ረ ጃ**

1የደምግፍትብቻ □ አዎ □ አይ

2 የደም ግፍት ከስ□ር ጋር □ አዎ □ አይ

3. ስካር ብቻ □ አዎ □ አይ

አዎ ከሆኔ የትኛዉ . □ አንደኛዉ □ ሁለተኛዉ

4.1ከስካርጋርየተያያዙችግሮች □ የኩላልትችግር □የእይታችግር □የአእምሮችግር

□የለም

5 ከልብጋርየተያያዙችግሮች

□ **Coronary artery disease** ከሆነ በየትኛዉ መ ንገድ ተለየ.....1

A clinical diagnosis of stable angiography……..1

B history of myocardial infarction……..2

C echocardiographic evidence of coronary artery disease……3

□ **Ischemic Stroke**፣ከሆነየትኛዉ ……2

A) Embolic……..1

B) Thrombotic…….2

□ **Peripheral Aterialism, ከሆነ በየት ኛ ዉ መ ንገድ ተለየ.....3**

A Doppler of ultrasound of lower extremities artery…….1

B angiography……..2