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Evaluation of Lipid Profiles and Hematological Parameters in Hypertensive Patients: A Cross Sectional Study at Debre Markos Referral Hospital, North West Ethiopia

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A Master's thesis submitted to the department of medical Biochemistry, school of Graduate Studies, Addis Ababa University in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of medical Biochemistry

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This is to certify that the dissertation prepared by Alemu Gebrie, entitled: ***Evaluation of Lipid Profiles and Hematological Parameters in Hypertensive Patients: A Cross Sectional Study at Debre Markos Referral Hospital, North West Ethiopia,*** 2016/17 and Submitted in partial fulfillment of the requirements for the degree “Master of Science in Biochemistry” in the department of medical Biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this research paper entitled: ***Evaluation of Lipid Profiles and Hematological Parameters in Hypertensive Patients: A Cross Sectional Study at Debre Markos Referral Hospital, North West Ethiopia***, 2016/17 is my original work and has not been presented for any degree in any other university, and that all sources of materials used for the research have duly been acknowledged.

Alemu Gebrie

Signature_____________________

Date________________________

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis Of Variance
BMI	Body Mass Index
BP	Blood Pressure
CVD	CardioVascular Disease
EDTA	EthyleneDiamineTetraacetic Acid
HDL-C	High Density Lipoprotein Cholesterol
HTN	Hypertension
JNC	Joint National Committee
LDL-C	Low Density Lipoprotein Cholesterol
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
NCEP-ATP	National Cholesterol Education Program Adult Treatment Panel
PEG	Polyethylene Glycol
RBC	Red Blood Cell
TC	Total Cholesterol
TG	Triglyceride
US	United States
WBC	White Blood Cell
WHO	World Health Organization

ABSTRACT

***Introduction**: Hypertension and dyslipidemia are the two coexisting and synergizing major risk factors for cardiovascular diseases. The cellular constituents of blood affect the volume and viscosity of blood, thus playing a key role in regulating blood pressure. Overweight and obesity are key determinants of adverse metabolic changes including increase in blood pressure.*

***Objective:** The aim of the present study was to evaluate lipid profiles and hematological parameters in hypertensive patients at Debre Markos Referral Hospital, North West Ethiopia.*

***Materials and methods**: Hospital based cross-sectional study was conducted in 100 hypertensive patients at Debre Markos Referral Hospital. The required amount of blood was withdrawn from the patients by health care professionals in the hospital for the immediate automated laboratory analysis of the blood sample. Data were collected on sociodemographic factors, anthropometric measurements, blood pressure, lipid profiles and hematological parameters.*

***Result:** The mean serum levels of Triglyceride (TG), Total Cholesterol (TC) and Low Density Lipoprotein (LDL) were significantly higher than their respective cut-off values in the hypertensive patients. 54%, 52%, 35%, 11% of the hypertensive patients had abnormal LDL, TC, TG and High Density Lipoprotein (HDL) levels respectively. Higher levels ($p<0.05$) of LDL, hemoglobin and Red Blood Cell (RBC) count were observed in the hypertensive patients whose blood pressure had been poorly controlled than the controlled ones. Waist circumference had significant positive association with the serum levels of TC and White Blood Cell (WBC) count ($p<0.05$).*

***Conclusion:** The hypertensive patients in this study had high prevalence of lipid profile abnormalities and poorly controlled blood pressure which synergize in accelerating other cardiovascular diseases. Some hematological parameters like RBC count are also increased as do the severity of hypertension.*

***Key words:** Hypertension, Lipid profiles, Hematological parameters, Anthropometric indicators*

1. INTRODUCTION

1.1. Overview of hypertension

Hypertension (HTN), for clinical purposes, can be defined as blood pressure more than 140/90 mm Hg as per US Seventh Joint National Committee on Detection, Evaluation and Treatment of Hypertension (JNC VII). BP values increase with age, and hypertension (persistently elevated BP values) is very common in the elderly. It is estimated that more than 26% of the population are with hypertension worldwide. Hypertension affects approximately 25% of the adult American population. The lifetime risk of developing hypertension among those 55 years of age and older who are normotensive is 90% (Chobanian *et al*., 2003).

Even though different risk factors have been identified for the development of hypertension (Whelton *et al*., 2002), its pathophysiologic etiology is still not fully understood in most (90%) patients (essential or primary hypertension). This form of hypertension cannot be cured, but it can be treated or controlled. However, a small percentage of patients have a specific cause for their hypertension (10%) i.e. secondary hypertension. There are many potential secondary causes that either are concurrent medical conditions or are endogenously induced. If the cause can be identified, hypertension in these patients can potentially be cured (Dipiro *et al*., 2014).

Blood pressure can be classified into one of the four categories: normal, prehypertension, stage 1 hypertension and stage 2 hypertension. Prehypertension is not considered as a disease, but identifies those who are likely to progress to stage 1 or stage 2 hypertension in the future. Blood pressure classification in adults (18 years and above) is based on the average of two or more properly measured blood pressure readings from two or more clinical visits (see **Table 1**). The overall classification is determined based on the higher of the two blood pressures, if the diastolic blood pressure and systolic blood pressure values fall into different classes (Chobanian *et al*., 2003).

Table 1: Classification of blood pressure in adults (>18 years old) (Chobanian *et al*., 2003)

Classification	Systolic BP (in mmHg)	Diastolic BP (in mmHg)
Normal	<120 and	<80
Prehypertension	120-139 or	80-89
Stage I HTN	140-159 or	90-99
Stage II HTN	>160 or	>100

HTN is known as the "silent killer" because it typically has no warning signs or symptoms, and many people do not know they have it. Frequently, the only sign of essential hypertension is elevated BP. The rest of the physical examination may completely be normal. However, a complete medical evaluation (a comprehensive medical history, physical examination, and laboratory and/or diagnostic tests) is recommended after diagnosis to (a) identify secondary causes, (b) identify other cardiovascular disease (CVD) risk factors or comorbid conditions like diabetes that may define prognosis and/or guide therapy or control, and (c) assess for the presence or absence of hypertension-associated target-organ damage (s) (Chobanian *et al*., 2003).

Various factors such as genetic, environmental, psychosocial, and inflammatory factors have been implicated in the development of hypertension (Tomson and Lip, 2005). The risk factors include health conditions, lifestyle, and family history. Some risk factors, such as family history, cannot be controlled. However, there are risk factors such as physical activity and diet that can be controlled to decrease a patient's likelihood of developing HTN (Dipiro *et al*., 2014). Therefore, gaining knowledge about factors that are associated with a blood pressure increase is imperative.

Many recent guidelines on the diagnosis and management of hypertension focus that total CVD risk should be quantified so that the type and intensity of treatment can be tailored to the degree of overall risk rather than the level of BP elevation alone. This approach maximizes the cost-effectiveness of hypertension management. The starting point of this therapeutic approach is the search for, and identification of, the various CVD risk factors (WHO, 2003). Hypertension is also one of the major manifestations of the group of clinical abnormalities that characterize metabolic syndrome found in 30 to 40% of hypertensive individuals (Marchi-Alves *et al*., 2012).

1.2. Literature review

1.2.1. Serum lipid profiles with respect to hypertension and CVD

High blood pressure is regarded as one of the most important underlying causes of cardiovascular diseases in our globe. It is usually related with other cardiovascular risk factors such as dyslipidemia (abnormal levels of lipids and lipoproteins in the blood), diabetes, and obesity. The presence of these cardiovascular risk factors and the resulting endothelial dysfunction may play a role in the pathophysiology of hypertension (Oparil *et al*., 2003). Evidence suggests that hypertension may share a similar pathophysiology with cardiovascular disease (CVD). Therefore, dyslipidemia, which is a strong predictor of CVD, may also be important to predict incident hypertension (Halperin *et al*., 2006).

Biochemically, complexes of variable proteins and lipid compositions called lipoproteins are responsible for transport of lipids throughout the body. The plasma lipoproteins are spherical macrobiomolecular substances made up of lipids and specific proteins called apolipoproteins or

Apo proteins. The lipoprotein complexes include high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low-density lipoproteins (VLDL), and chylomicrons (CM) (**Table 1**). The complexes play key roles in maintaining their component lipids soluble while they transport them in the blood and providing an efficient mechanism for transporting their lipid contents to and from the body tissues.

In humans, the transport system is less efficient than in other animals and, as a consequence, humans are vulnerable to gradual deposition of lipid substances (dyslipidemia) —especially cholesterol—in body tissues. This is a potentially life-threatening occurrence when the lipid deposition contributes to plaque formation, causing the narrowing of blood vessels (atherosclerosis)(Harvey and Ferrier, 2011).

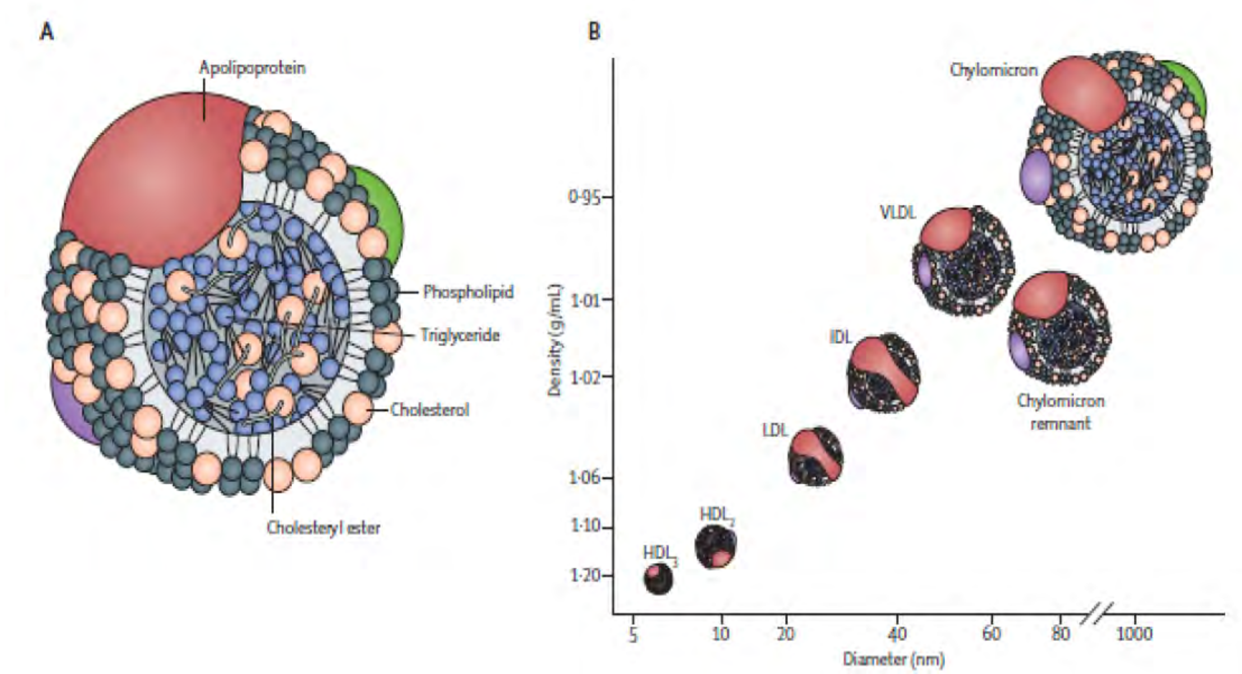

Figure 1: Structure, types and densities of lipoproteins in the blood from Braun Wald's Heart Disease: (Mann *et al*., 2015)

LDL particles, associated with the Apo lipoprotein molecule Apo lipoprotein B, contain much less triacylglycerol than their VLDL predecessors, and have a high concentration of cholesterol and cholesteryl esters. The primary function of LDL particles is to provide cholesterol to the peripheral tissues where they are internalized through the LDL receptor by receptor-mediated endocytosis. Genetic defects that result in loss of function of LDL receptor cause inherited hyperlipidemias (Mooradian, 2009, Harvey and Ferrier, 2011).

Increased concentrations of serum LDL cholesterol are associated with an increased risk of myocardial infarction and stroke, and reaction of LDL-C with reactive oxygen species is an early step in atherosclerotic plaque formation. Individuals who inherit one copy of a defective LDL receptor-related gene (heterozygous Familial Hypercholesterolemia), left untreated, often have myocardial infarctions in their 30s and 40s. If a person is homozygous for these mutations (homozygous Familial Hypercholesterolemia), they have extremely high serum LDL-C levels and can have myocardial infarctions in their late teens and early 20s (Mooradian, 2009).

High density lipoprotein (HDL-C) contains Apo lipoprotein A-1. Formation of HDL occurs in the liver and intestine, which both synthesize and secrete ApoA-I. Shortly after secretion as a lipid poor protein, ApoA-I interacts with the cholesterol–phospholipid transporter ABCA1 (ATP Binding Cassette A1) expressed by hepatocytes and enterocytes to acquire lipids, thereby generating a nascent HDL particle (Grummer and Carroll, 1988).

Plasma lipid profiles are measured for cardiovascular risk prediction and have now become almost a routine test. The test includes four basic parameters: total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol. Worldwide, there is broad variation in serum lipid profile patterns among different population groups. Increased serum levels of TC, TG, LDL-C, and decreased serum HDL level are known to be associated with major risk factors for CVD. Dyslipidemia, comprising altered ratio of high TC level and isolated evaluation of the LDL-C or TG, is usually associated with increased blood pressure (BP) levels. There is a strong relationship between total LDL cholesterol concentrations and CVD risk (Choudhury *et al*., 2014).

Although hypertension and dyslipidemia are widely recognized risk factors for cardiovascular diseases, the pathophysiology of the association between them is still not completely understood. Several mechanisms have been postulated to explain this association. Atherosclerosis – caused in great part by dyslipidemias – leading to structural changes that result in the decreased elasticity of large arteries, is generally viewed as the principal pathophysiologic alteration contributing to the development of arterial hypertension in the elderly (Oparil *et al*., 2003).

Plethora of studies have prospectively examined if increased lipid levels are correlated with the afterward development of hypertension in middle age adults. In most studies, high-density lipoprotein cholesterol (HDL-C) levels show an independent and inverse relation with the development of hypertension (Halperin *et al*., 2006, Simone *et al*., 2006, Laaksonen *et al*., 2008). Increased triglycerides levels (Laaksonen *et al*., 2008) higher total cholesterol (total-C) (Sesso *et al*., 2005) increased non-HDL cholesterol (non- HDL-C) and higher levels of low density lipoprotein cholesterol (LDL-C) have been found to be associated with an increased risk of hypertension in some studies, but not in all (Simone *et al*., 2006).

1.2.2. Hematological profiles and hypertension

The cellular components of blood contribute to the viscosity and volume of blood, thus playing a vital role in regulating blood pressure. Hematological parameters (full blood count) which include red blood cell (RBC) count, hemoglobin, hematocrit, platelet count, white blood cell (WBC) count and RBC indices are the backbones of any laboratory evaluation and their abnormal values may be associated with various pathological conditions.

Red blood cell count, hematocrit, and hemoglobin provide estimates of red blood cell number, red cell proportion, and hemoglobin concentration, respectively, in a volume of blood. In view of a predominantly thrombotic nature of the complications of hypertension, various rheological and hemostatic factors have been hypothesized to play a role in the pathogenesis of hypertension and other cardiovascular diseases (Yamasaki *et al*., 2005).

Although it is yet to be well characterized, studies have shown that white blood cell (WBC) count has been found to be associated with hypertension and its complications (Karthikeyan and Lip, 2006). Inflammation may contribute to increasing resistance of microvascular capillary, initiation of platelet aggregation, increased levels of catecholamines, and there is considerable evidence of an association between inflammation and hypertension (Bautista *et al*., 2001).

With respect to the relationship between WBC count and hypertension, one population-based study found an association between elevated WBC count and incident hypertension in a predominantly white population, with the risk ratio of hypertension being directly related in a dose-dependent manner to increasing tertiles of WBC count (WBC count tertiles 1–3 has relative risks (RR) of hypertension of 1.0, 1.2, 1.7; $P<0.01$). This association appeared to be independent of smoking and other cardiovascular risk factors (Shankar *et al*., 2004).

In other study done in Japan it was observed that elevated WBC count, especially neutrophil count, was significantly associated with an increased risk of developing hypertension among Japanese men and women, although the relative risks were modest (Tatsukawa *et al*., 2008).

Hemoglobin is most important determinant of whole blood viscosity (Simone *et al*., 1990). Studies have shown that the concentrations of hemoglobin increased with hypertension in humans. However, only a limited number of large-population based studies have shown a link between hemoglobin concentration and blood pressure. In another study conducted among unselected public employees who did not receive any medication, hemoglobin concentration was significantly associated with hypertension (Kawamoto *et al.*, 2012, Shimizu *et al.*, 2014).

In a recent study involving a large cohort of blood donors who were relatively healthy, hemoglobin concentrations were positively associated with both systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Atsma *et al*., 2012). The recent Japanese study also included non-anemic subjects and found that positive association between the hemoglobin concentrations and the risk of hypertension was confined to participants with a body mass index (BMI) of > 25 kg/m^2 (Shimizu *et al*., 2014). Researchers also reported that three erythrocyte parameters (RBC, hemoglobin, and hematocrit) were found to be associated with hypertension in their cohort study (Wu *et al*., 2013).

Hematocrit, the proportion of blood volume occupied by red blood cells, determines blood viscosity, regulates peripheral vascular resistance (PVR) and therefore, in principle, regulates blood pressure. The association between hematocrit and pre-hypertension was less significant among individuals with high LDL-C levels compared to those with lower LDL-C levels. Moreover, LDL-C and hematocrit showed an additive pattern in elevated blood pressure (Liu *et al*., 2015).

There is mounting evidence that metabolic syndrome affects hematologic counts. Obesity is found to be associated with higher hemoglobin levels and elevated WBC counts. Variability of blood counts and differentials was associated with obesity, hypertension, dyslipidemia, and glucose intolerance. Hematologic changes associated with parameters of metabolic syndrome have also been demonstrated in studies (Kawamoto *et al*., 2012, Vuong *et al*., 2014).

1.2.3. Anthropometric indicators and hypertension

As reported by several epidemiological studies from different populations there is a significant association between different anthropometric indicators and blood pressure levels (Stamler, 1991, Guagnano *et al*., 1994, Gupta and Mehrishi, 1997 , Kaufman *et al*., 1997, Olatunbosun *et al*., 2000, Bose *et al*., 2003, Shanthirani *et al*., 2003). Some anthropometric indexes or measures, like body mass index (BMI), and other measures of body fat distribution have been utilized in most of the studies to analyze the relation between cardiovascular risk factors and adiposity (Han *et al*., 1995, Olatunbosun *et al*., 2000, Guagnano *et al*., 2001, Sargeant *et al*., 2002, Belahsen *et al*., 2004).

Most of these studies have shown direct relationships between anthropometric measures and the risk of cardiovascular disease (Stamler, 1991, Guagnano *et al*., 1994, Kaufman *et al*., 1997, Kadiri *et al*., 1999, Okosun *et al*., 1999, Olatunbosun *et al*., 2000, Yekeen *et al*., 2003). These associations between body fatness using different indexes have been consistently observed, but remain poorly understood (Kaufman *et al*., 1997) and the mechanistic explanations for the phenomenon are still being debated, and no biological model of the process has been established (Han *et al*., 1995).

Patterns of lipid abnormalities among Ethiopians and their relative impact on cardiovascular risk have not yet been well characterized. Low HDL is increasingly recognized as an independent risk factor for adverse CVD outcomes, irrespective of levels of LDL-C. Although sporadic reports suggest that the prevalence of low HDL-cholesterol is substantial, detailed data is lacking on the true prevalence of this condition among patients receiving treatment for dyslipidemia (Karthikeyan *et al*., 2009). Little is known about the anthropometry, lipid profiles and hematological parameters–blood pressure relationship in Ethiopian subjects, while no data seems to be available on the relationship between body-fat distribution and the risk of hypertension in the same population.

1.3. Statement of the problem

Cardiovascular diseases including hypertension are increasing worldwide. This increase is causing a major concern in resource limited countries like Ethiopia. In 2000, about 1 billion people (26.4% of adults) were estimated to have hypertension worldwide and this is likely to increase to over 1.5 billion by 2025 as a result of aging population in many developed countries, and an increasing incidence of hypertension in developing countries (Cornier *et al*., 2008). In Ethiopia, it has approximately been estimated that about 35.2% of the population are suffering from hypertension (WHO, 2011).

Several risk factors (modifiable and non-modifiable) play a role in the progression of hypertension (Leone, 2011). In an investigation on the different genetic and environmental risk factors of hypertension, studies showed that age, sex, hyperlipidemia, diabetes, alcohol consumption, high Body Mass Index (BMI), sodium intake and others were associated with hypertension. An excessive daily intake of saturated fats, cholesterol, and subsequent disturbance of lipid profiles leading to hypertriglyceridemia and hypercholesterolemia are associated with obesity and, consequently, hypertension (Kotsis *et al*., 2010).

Hypertension and dyslipidemia, co-existing in 15 to 31%, are the two major risk factors for cardiovascular diseases (CVD) and account for more than 80% of deaths and disability in low- and middle-income countries (Reddy, 2004). These risk factors have an adverse effect on the vascular endothelium, which results in enhanced atherosclerosis resulting in CVD (Dalal *et al*., 2012). Abnormalities in serum lipid levels can be recognized as major modifiable CVD risk factor and has been identified as a risk factor for essential hypertension giving rise to the term dyslipidemic hypertension (Halperin *et al*., 2006).

Hypertension is not the only determinant of cardiovascular damage and the propensity of BP poorly controlled hypertensive patients to develop target organ damage is markedly influenced by coexisting risk factors. Among these factors lipoproteins are fundamental to the atherosclerotic process and greatly affect the impact of hypertension on development of target organ damage and therefore on cardiovascular morbidity and mortality (Srinivaspai *et al*., 2014).

In addition, there are number of disputes in various studies with respect to variability of hematological parameters in patients with hypertension and normotensive subjects. The pathophysiology of hypertension is multifactorial which is affected by sympathetic over activity contributing to changes in hematological parameters like hematocrit, viscosity and hypercoagulability of blood. These factors vary the kinetics of blood flow acting as contributory risk factor for coronary artery diseases, stroke and thromboembolism (Al-Muhana *et al*., 2006). Thus, the hematological parameters will give an insight to prognosis of the disease as well.

Although different studies have been done on lipid profiles as well as hematological parameters in hypertensive patients in different parts of the world (Osuji and Omejua, 2012, Tachebele *et al*., 2014, Divya and Ashok, 2016), there are no ample data on the condition in Africa especially Ethiopia. To the best of my knowledge, there are no reports on the evaluation of lipid profiles as well as hematological parameters particularly in the study area.

Worldwide, there is broad variation in serum lipid profile patterns among different population groups. Therefore, evaluation and monitoring of modifiable risk factors can be beneficial to reduce CVD morbidity and mortality of the patients. The present study will evaluate and examine lipid profiles and hematological parameters and other associated risk factors in hypertension in the study area and give recommendations so as to minimize CVD morbidities and mortalities.

1.4. Rationale and significance of the study

CVD is the primary cause of disability and death worldwide, and a great majority of CVDs are associated with dyslipidemia. The problem is disturbing and causing a major concern in resource limited countries like Ethiopia. Unfortunately, due to socioeconomical and other factors patients come to health facilities at advanced stage of cardiovascular diseases including hypertension in Ethiopia. Therefore, evaluating and examining serum lipid parameters and hematological profiles, and other associated risk factors in patients with hypertension in our setup will significantly be important to help shape clinical as well as public health care of the patients and the population. In addition, the results obtained from this study is expected to pave the way for further related studies to be broadly and extensively done.

2. OBJECTIVES

2.1. General objective

- To evaluate lipid profiles and hematological parameters in hypertensive patients at Debre Markos Referral Hospital, North West Ethiopia

2.2. Specific objectives

- ☞ To evaluate the serum lipid profiles in male and female hypertensive patients with good and poor blood pressure control
- ☞ To evaluate hematological parameters (complete blood count) in male and female hypertensive patients with good and poor blood pressure control
- ☞ To assess anthropometric parameters in the hypertensive patients
- ☞ To examine the associations of sociodemographic factors, blood pressure and anthropometric parameters with lipid profiles and hematological parameters in the hypertensive patients

3. MATERIALS AND METHODS

3.1. Study area and period

The study was conducted from October, 2016 to January, 2017 at Debre Markos Referral Hospital, Debre Markos. Debre Markos, the capital of East Gojjam Administrative Zone is located in the north west of the capital city of Ethiopia, Addis Ababa at a distance of 300Kms and 265 kms to the capital of Amhara Nation Regional State, Bahir Dar. Debre Markos Referral Hospital is found in this town. It was established in 1957 E.C by Emperor H/Selassie on the area of 30,020 m^2. The hospital provides health service to more than 3.5 million people. Currently about 100 health centers and four district hospitals are available in the catchment area of the referral hospital.

3.2. Study design

Hospital based cross-sectional study was conducted to evaluate the serum levels of lipid profiles and hematological parameters among hypertensive patients at Debre Markos Referral Hospital, Debre Markos.

3.3. Population

3.3.1. Source population

The source population for this study was all hypertensive patients attending at Debre Markos Referral Hospital.

3.3.2. Study population

The study population for this study was all hypertensive patients attending at Debre Markos Referral Hospital in the time interval of the study period.

3.4. Inclusion and exclusion criteria

3.4.1. Inclusion criteria

- All hypertensive patients attending at the hypertension out-patient department of the hospital during data collection period were included in the study.

3.4.2. Exclusion criteria

- Age < 20 years
- Age > 70 years
- Patient on lipid lowering medications
- Thyroid disease
- Pre-eclampsia/eclampsia
- Patients with hematological derangement
- Diabetes mellitus

3.5. Sampling method and sample size determination

While purposive sampling technique was implemented to select the health care facility simple random sampling technique from registry book was used to get the calculated number of study participants in the study period. By using semi-structured questionnaire, the patients were selected that include all pieces of information of patients needed at study variables.

The sample size was determined based on prevalence of hypertension (19.6%) in Ethiopia as reported by systematic meta-analysis (Kibret and Mesfin, 2015), using single population proportion formula with a confidence interval (CI) of 95%).

$$n = \frac{\left(Z_{1-\alpha/2}\right)^2 P(1-P)}{d^2}$$

Where **n** is minimum sample size required; $\mathbf{Z_{1-\alpha/2}}$ is the standard normal variable at (1-α) % confidence level and α (level of significance), Usually 95% confidence level is used = 1.96; **P** is estimate of the prevalence rate of hypertensive patients in the population; **d** is the margin of sampling error tolerated, assume to be 0.05. N= 400, So, n after adjustment is approximately 153 patients, but due to budget constraint only 100 patients were enrolled.

3.6. Variables

3.6.1. Dependent variables

- Serum total cholesterol concentration (TC)
- Serum triglyceride concentration (TG)
- High density lipoprotein (HDL) cholesterol concentration
- Low density lipoprotein (LDL) cholesterol concentration
- Hemoglobin
- Hematocrit
- RBC
- WBC
- Platelets
- RBC indices

3.6.2. Independent variables

- ➢ Sociodemographic factors

- Family history
- Clinical and behavioral factors
- Anthropometric indicators

3.7. Blood sample and data collection procedures

After the study participants had been asked for their consent to be interviewed and to give sample blood, about 5 ml blood was withdrawn from the study participants, who had fasted overnight. The sample was collected by qualified health care professionals in the hospital for the immediate laboratory analysis of the blood sample. In addition, the questionnaire was filled by face to face interview and some anthropometric indicators were also assessed and measured side by side as well.

Blood collected in appropriate tubes was allowed to stand for 30 minutes at room temperature to allow complete clotting and clot retraction. Samples were then centrifuged at 3500 rpm for 15 min to extract serum. The serum extracted was then used to determine the levels of TC, HDL-cholesterol and triglycerides.

LDL-cholesterol was calculated using the Friedwald formula (Friedewald *et al*., 1972). About 2ml of the blood was collected in EDTA coated tubes and hematological profiles were determined for all samples using a hematological analyzer (ACT-8 coulter Electronies). Safety precautions were taken while handling blood and disposing it.

3.8. Test principles of the laboratory analytes

3.8.1. Serum total cholesterol concentration

A commercial kit developed by Coxon and Schaffer was used to estimate serum total cholesterol concentration (Coxon and Schaffer, 1971). Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. Desirable or normal cholesterol levels were considered to be those below 200 mg/dL. The reaction sequence is as follows:

Cholesteryl ester + H_2O $\xrightarrow{\text{cholesteryl ester hydrolase}}$ cholesterol + fatty acid

Cholesterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ cholest- 4-en-3-one + H_2O_2

$2H_2O_2$ + 4-aminophenazone + phenol $\xrightarrow{\text{peroxidase}}$ 4-(p-benzoquinone- monoimino)-phenazone + 4 H_2O

3.8.2. Serum triglyceride concentration

A commercial kit developed from Cromatest® Cholesterol MR, Linear chemicals SL, Barcelona, Spain was used to estimate serum triglyceride concentration (Allain *et al.*, 1974). Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

Triglycerides + 3H2O $\xrightarrow{\text{lipase}}$ glycerol + fatty acids

Glycerol + ATP $\xrightarrow{\text{glycerokinase}}$ glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O2 $\xrightarrow{\text{glycerophosphate oxidase}}$ dihydroxyacetone phosphate + H2O2

H2O2 + 4-aminophenazone + 4-chlorophenol $\xrightarrow{\text{peroxidase}}$ 4-(p-benzoquinone-monoimino)-phenazone + 2H2O + HCl.

Desirable or normal fasting triglyceride levels are considered to be those below 200 mg/dL, and are further categorized as Borderline, 200-400 mg/dl; High, 400-1,000 mg/dl; and Very High (> 1000 mg/dl).

3.8.3. High density lipoprotein (HDL) cholesterol concentration

A commercial kit developed from Cromatest® Cholesterol MR, Linear chemicals SL, Barcelona, Spain was used to estimate serum HDL concentration. HDL was measured directly in serum. The basic principle of the method is as follows. The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-cholesterol is detected under the assay conditions.

The method uses sulfated alpha-cyclodextrin in the presence of Mg^{+2}, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL-cholesterol measurement. The reactions are as follows:

a. ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 $\longrightarrow$ soluble non-reactive complexes with apoB-containing lipoproteins

b. HDL-cholesteryl esters $\xrightarrow{\text{PEG-cholesteryl esterase}}$ HDL-unesterified cholesterol + fatty acid

c. Unesterified chol + O_2 $\xrightarrow{\text{PEG-cholesterol oxidase}}$ cholestenone + H_2O_2

d. H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'_succinyl ethylene diamine + H2O + H^+ $\xrightarrow{\text{peroxidase}}$ qunoneimine dye + H_2O

Absorbance was measured at 600 nm. A low HDL-cholesterol concentration is considered to be a value below 45 mg/dl. HDL-cholesterol values are also used in the calculation of LDL-cholesterol (as shown below)

3.8.4. Low density lipoprotein (LDL) cholesterol concentration

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low density lipoproteins (VLDL), LDL and HDL. Total cholesterol is the sum of VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol. LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship: *LDL-cholesterol = Total cholesterol - HDL-cholesterol – (TG/5)* where TG/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dl. Desirable levels of LDL-cholesterol are those below 100 mg/dl in adults.

3.8.5. Determination of hematological parameters

The Coulter method, automated hematology analyzers, was used to accurately count and size cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. The number of pulses correlates to the number of particles. The height of the electrical pulse is proportional to the cell volume.

Hemoglobin, hematocrit, platelets, RBC count, WBC count, RBC indices were determined. The RBC count, WBC count, and Platelets were determined by the principle of electronic impedance. The hemoglobin which was freed by the lysis of RBCs was combined with potassium cyanide which the forms a cyanmethemoglobin compound. The absorbance was then measured by spectrophotometry at 550nm wavelength. MCV was calculated directly from RBC histogram. MCH was calculated from hemoglobin level and RBC count. In addition, MCHC was calculated according to the hemoglobin and hematocrit values. The hematocrit was measured as a function of the numeric integration of MCV (Carberand *et al*., 1999)

$$MCV = \frac{Hematocrit}{RBC\ count}$$

$$MCH = \frac{Hemoglobn}{RBC\ count}$$

$$MCHC = \frac{Hemoglobin}{Hematocrit}$$

3.9. Anthropometrical measurement procedure

The weight of the hypertensive patients was measured using a standard balance, and the height was measured by using a height measuring device attached to the balance.

Body Mass Index (BMI) was then calculated from the body weight (kg) and height (meter) as follows: BMI = Weight (in kg) / (Height in m)^2 (Tambe *et al*., 2010). Using the WHO classification (WHO, 1997), four categories of BMI can be identified as follows: underweight, <18.5 kg/m^2; normal, >18.5–24.9 kg/m^2; overweight, >25.0–29.9 kg/m^2; and obesity, >30 kg/m^2. The participants' ages were also recorded.

Waist circumference and hip circumference of the patients were also measured. Waist circumference was measured over light clothing at the level halfway between the iliac crest and the costal margin in the mid-axillary line after exhaling, when the lungs are at their functional residual capacity, with the subject in standing position with the body weight evenly distributed across the feet. Hip circumference was measured over light clothing at the level of greater trochanters with the subject in standing position and both feet together (Tambe *et al*., 2010).

Two consecutive recordings were made for each site to the nearest 0.5 cm using a non-stretchable fiber measuring tape on a horizontal plane without compression of skin. The mean of two sets of values was used (Luepker, 2004). Waist to hip ratio was calculated by dividing waist circumference by hip circumference (Tambe *et al*., 2010).

While the cut-off point considered for waist circumference (WC) was >80 cm for females and >90 cm for males to define overweight, the cut-off taken for waist to hip ratio was >0.8 for females and >0.9 for males as per the criterion of the WHO (WHO, 2008).

3.10. Data quality control and management

- The data collection questionnaire was well prepared and all variables were filled on the data extraction format daily.
- All the laboratory procedures were handled by professional laboratory technologists.
- All the tests were standardized and automated.

3.11. Data processing and analysis

After checking for completeness and cleaning, processing and analysis of the data obtained from laboratory analyses of the blood samples and questionnaires was performed by coding and entering the data into EpiData statistical software version 3.1 and then exporting the entered data to SPSS software version 23 package and the different variables were tested and analyzed. Simple descriptive statistics were used to present the sociodemographic and clinical characteristics of the study subjects. While Chi-square ($\chi2$) tests were used to compare categorical variables, continuous variables were presented as mean ± standard deviation and were compared using the student t-tests for groups. Other associations were performed with Pearson's correlation coefficient as well as multiple linear regression analysis. A p-value of <0.05 at 95% confidence level was considered to be statistically significant in all the analyses.

3.12. Ethical consideration

Before starting data collection and preliminary study, ethical clearance letter with reference number SOM/DRERC/BCHM031/2009 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. Collaboration letter for data collection was also obtained from Debre Markos referral hospital. The objective of the study was briefly clarified and explained for each participant, before enrolling any of the eligible study participants. Samples and data were collected after informed consent had been obtained from the study participants. Confidentiality, anonymity, neutrality, accountability and academic honesty was maintained throughout the study, for example, by using codes. The findings of the study will be disseminated for health care professionals and other concerned bodies for better care of the hypertensive patients than ever.

3.13. Operational definitions

Dyslipidemia: synonymous with hyperlipidemia or lipid abnormalities is abnormally elevated levels of any or all lipids and or lipoproteins in the blood. By the same token it is a defect in lipoprotein metabolism; example, increased cholesterol, increased triglyceride, increased low density lipoprotein, and decreased high density cholesterol.

Controlled blood pressure (hypertension): blood pressure that is controlled by antihypertensive drug (s) which used in the sample hypertensive patients were (Enalapril, Nifedipine, Hydrochlorothiazide, Amlodipine) or pharmacological and non-pharmacological treatment, i.e., systolic blood pressure is lower than 140 mmHg and diastolic blood pressure is lower than 90 mmHg.

Uncontrolled or poorly controlled blood pressure: blood pressure not well controlled despite the antihypertensive drugs prescribed, i.e., systolic blood pressure is higher than 139 mmHg and/or diastolic blood pressure is higher than 89 mmHg.

Anthropometric indicators: parameters for the measurement of the human body and its individual parts thereby yielding a quantitative index of their variability. They include age, height, weight, body mass index, waist circumference, waist to hip ratio etc.

4. RESULTS

4.1. Sociodemographic characteristics of the patients

Theis study enrolled 100 sample hypertensive patients, 45 (45%) females and 55 (55%) males. The average age of the hypertensive patients was 51.21 years ranging from 21 to 69 years. The majority of the hypertensive patients were found within in the age group of 40-59 years (**Figure 2**). Most of the patients in the study were married (71%), urban residents (78%), and above secondary school (41%) as far as educational status is concerned. While half of the patients 50 (50%) had history of alcohol drinking behavior, only 2 % of the hypertensive patients did smoke cigarette; and there were more male drinkers and smokers than females. In addition, most of the patients (55%) had history of performing different forms of physical activity (**Table 2**).

Table 2: Sociodemographic characteristics of the hypertensive patients at Debre Markos Referral Hospital, Ethiopia, October 2016 to January 2017

Variables		Males (n=55)	Females (n=45)	Total (n=100)
Age[a]		52.82 ±11.12	49.24 ±13.47	51.21 ±12.30
Marital status[b]	Single	1	9	10
	Married	47	24	71
	Divorced	4	5	9
	Widowed	3	7	10
Residence	Rural	16	6	22
	Urban	39	39	78
Educational status	Illiterate	19	12	31
	Up to 12	7	21	28
	Above	29	12	41
Smoking status	Yes	2	0	2
	No	53	45	98
Alcohol consumption	Yes	36	14	50
	No	19	31	50
Physical exercise	Yes	38	17	55
	No	17	28	45

[a]Age, continuous variable, is expressed as mean ± standard deviation; [b]for the rest of the variables, qualitative, the numbers are in percent out of the total 100 patients.

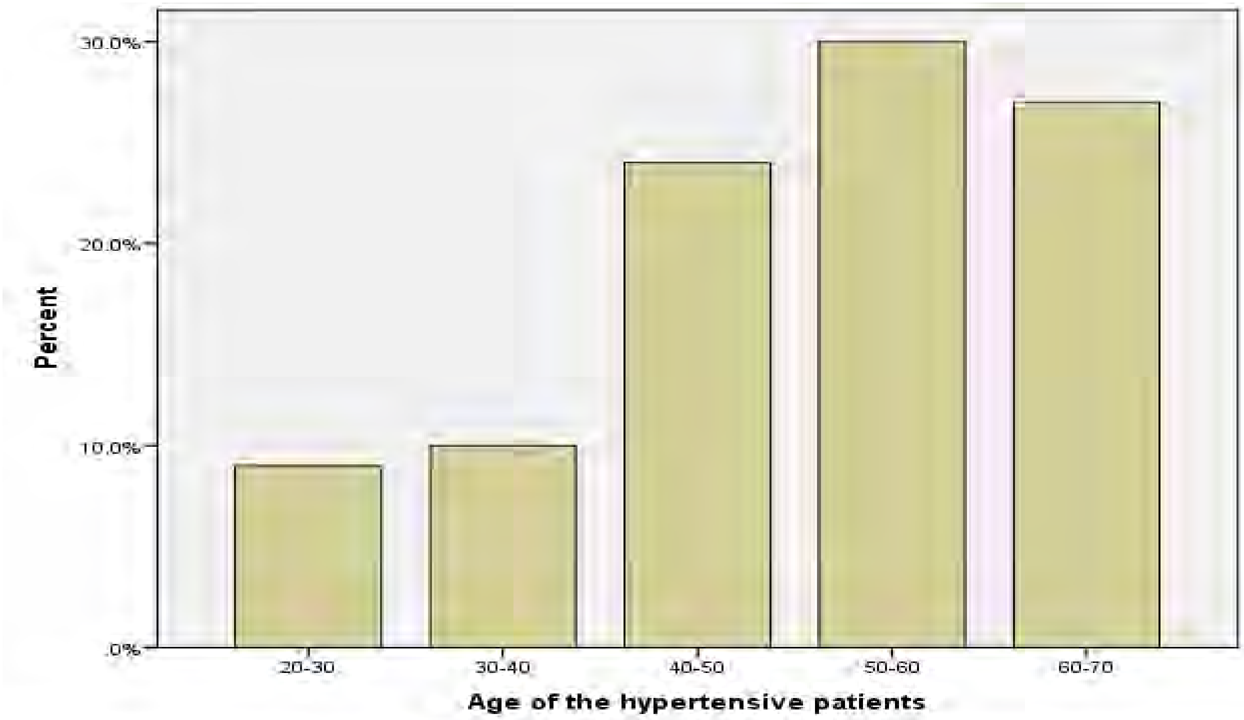

Figure 2: Age distribution of the hypertensive patients in years following chronic care at Debre Markos Referral hospital, Ethiopia, October 2016 to January 2017

4.2. Anthropometric and clinical features of the hypertensive patients

The present study revealed that the average BMI is high-normal (24.60 Kg/m^2) in the study participants. About 31% and 10% of the hypertensive patients were overweight and obese respectively. Females are affected more frequently than males (18 % overweight and 10% obese versus 13 % overweight and 0% obese). The study also showed that 36/55 males and 41/45 females had waist circumference greater than their respective cut-off values; and 49/55 males and all female hypertensive patients had Waist-to-hip ratio higher than the cut-off value (**Table 3**). Among the hypertensive patients 38% of them were found to have family history of hypertension, and more than half of the patients (62%) had uncontrolled blood pressure despite at least one antihypertensive drug prescribed for. Whereas mean systolic and diastolic blood pressures were found to be 138.18±17.857 and 84.55±9.193 mmHg respectively in males, the mean systolic and diastolic blood pressures of females were 146.22±29.641 and 87.33±17.633 mmHg respectively.

Table 3: Anthropometric and clinical features of the hypertensive patients at Debre Markos Referral Hospital, Ethiopia, October 2016 to January 2017

Characteristics		Males (n=55)	Females (n=45)	Total (n=100)
[a]Height (m)		1.6862± 0.08330	1.605±0.046	1.6498±0.07973
Weight (in Kg)		65.009±6.5287	68.500±9.6478	66.580 ±8.2259
[b]Body Mass Index(kg/m^2)		22.9569±2.69921	26.6082±3.83881	24.6000±3.72245
	<25	42	17	59
	25-30	13	18	31
	>30	0	10	10
Waist circumference (cm)		91.0545±8.87864	98.6667±14.1598	94.4800±12.10767
	< cut-off	19	4	23
	>cut-off	36	41	77
Waist-to-hip ratio		0.9558±0.05061	0.9482±0.05536	0.9524±0.05267
	< cut-off	6	0	6
	>cut-off	49	45	94
Family history of hypertension	Yes	15	23	38
	No	40	22	62
Duration of hypertension	< 1 year	8	3	11
	1-5 years	27	31	58
	>5 years	20	11	31
Blood pressure (mm Hg)	Controlled	22(124.5, 77.3)[e]	16(122.5,73.75)[e]	38[c]
	BP poorly controlled	33(147.3, 89.4)[e]	29(159.3, 94.8)[e]	62[d]
	Systolic[f]	138.18±17.857	146.22±29.641	141.80±24.095
	Diastolic[f]	84.55±9.193	87.33±17.633	85.80±13.646

[a]Continuous variables like height are expressed as mean ± standard deviation whereas [b]for the rest of the variables, qualitative, are expressed in percent out of the total patients. [c]systolic and diastolic BP (123.68±9.421 and 75.79±5.98); [d]systolic and diastolic BP (152.90±23.64 and 91.94±13.41), [e]values in bracket are the mean systolic and diastolic BP values respectively. [f]mean BP values are for all hypertensive patients (both BP controlled and BP poorly controlled).

4.3. Levels of lipid panels in the hypertensive patients

The mean levels of lipid profiles in BP controlled and BP poorly controlled male and female hypertensive patients are shown in **Table 4**. The result of this study showed that in the plasma of male BP controlled and BP poorly controlled hypertensive patients, the average total cholesterol levels were 193.42±54.91mg/dl and 227.00±34.07.57 mg/dl, and the levels of LDL-C were found to be 106.85±38.31 and 128.00±36.00 mg/dl respectively (see **Table 4**). In addition, the results of the present study showed that in the plasma of female BP controlled and BP poorly controlled hypertensive patients, the average TG levels were 148.88±45.59 mg/dl and 262.59±180.53 mg/dl, and the levels of LDL-C were found to be 92.00±33.36 and 127.72±57.56 mg/dl respectively (see **Table 4**). In both sexes LDL-C levels were significantly higher ($p<0.05$) in patients whose BP is poorly controlled than the controlled ones. However, patients with poor BP control as compared to good BP control had significantly higher levels ($p<0.05$) of TC in males and TG in females.

Table 4: Levels of lipid profiles in BP controlled and BP poorly controlled male and female hypertensive patients at Debre Markos Referral Hospital, Ethiopia, October 2016 to January 2017

Variables	Males (n=55)			Females (n=45)		
	BP controlled	BP poorly controlled	p-value	BP controlled	BP poorly controlled	p-value
TC	193.42±54.91	227.00±34.07.57	0.014*	184.88±55.54	216.66±62.28	0.096
TG	183.09±70.30	202.94±164.99	0.59	148.88±45.59	262.59±180.53	0.018*
LDL-C	106.85±38.31	128.00±36.00	0.045*	92.00±33.36	127.72±57.56	0.028*
HDL-C	65.33±17.90	61.36±21.88	0.46	72.00±19.90	59.75±19.49	0.053

[a]Values are expressed as mean ± standard deviation

Among 100 hypertensive patients, only 46 (46 %) of them had desirable level of LDL-C which is below 100 mg /dl, the cut-off value for the metabolite. The rest 54(54 %) had undesirable level of LDL-C i.e. greater than 100 mg /dl. On the other hand, while only 11 (11 %) of the hypertensive patients showed undesirable level of HDL-C cholesterol lipoprotein, in most of the patients (89 %) levels of serum HDL-C were found to be with in normal range which is below 45 mg/dl (**Figure 3**).

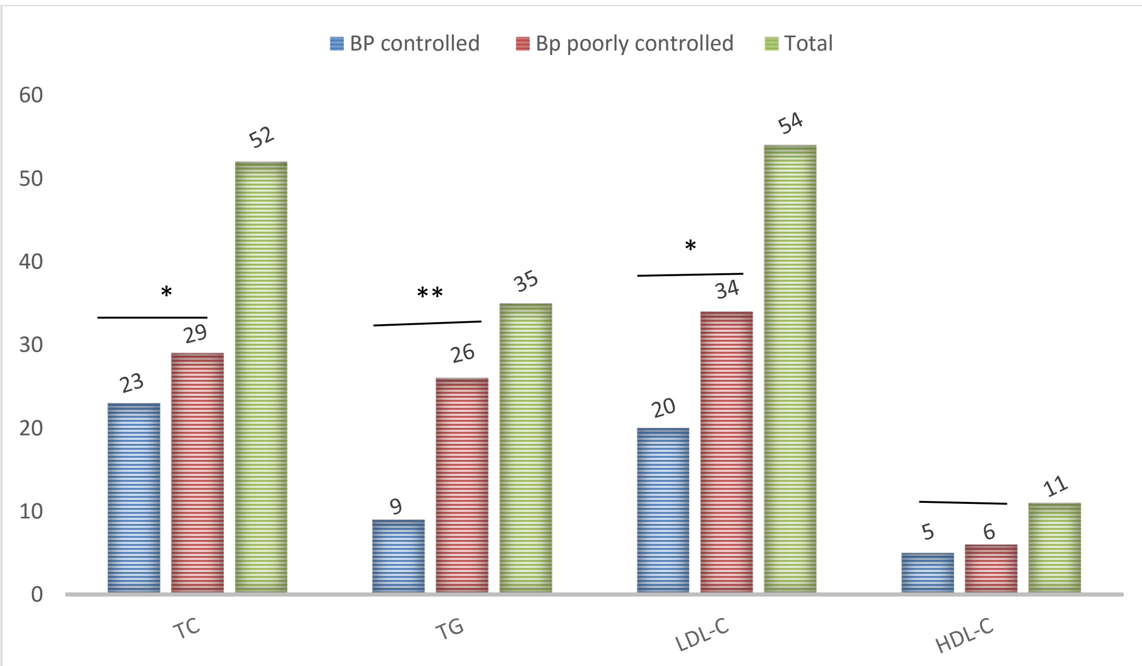

Figure 3: Percentage (absolute number) of BP controlled and BP poorly controlled hypertensive patients having abnormal levels of lipid profiles at Debre Markos Referral Hospital, Ethiopia, October 2016 to January 2017 (*P<0.05 **P<0.01)

In the sample hypertensive patients, 48(48 %) of them had normal serum total cholesterol level which is below 200 mg /dl, the cut-off level for the metabolite. But, the remaining patients 52(52 %) had abnormal level of total cholesterol (greater than 200 mg /dl). Whereas 65/100 (65 %) of the hypertensive patients had desirable level of serum triglyceride, in 35% of the patients, levels of serum triglyceride were found to be abnormally high which is above 200mg/dl.

The proportions of lipid profile abnormalities stratified by sex are depicted in the figure below (**Figure 4**). LDL-C and TC abnormalities were more prevalent in males as compared to their female counterparts.

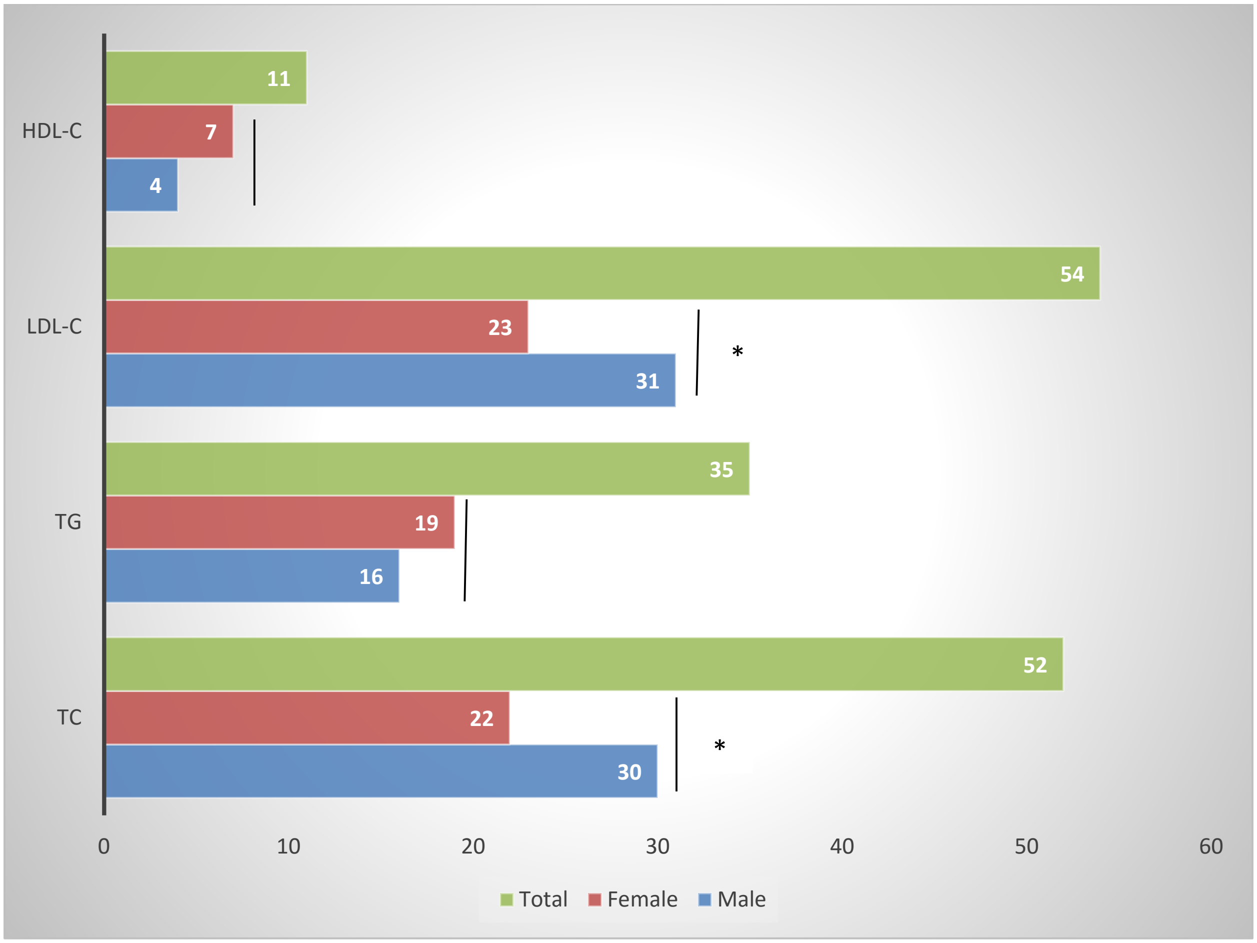

Figure 4: Percentage (absolute number) of hypertensive patients having abnormal levels of lipid profiles stratified by sex at Debre Markos Referral Hospital, Ethiopia, October 2016 to January 2017 (*P<0.05)

4.4. Levels of hematological parameters in hypertensive patients

The average levels of the hematological parameters are shown in **Table 5**. The study showed statistically significant elevation in WBC and RBC levels in BP poorly controlled male hypertensive patients as compared to BP controlled patients ($p<0.05$). Also, it showed statistically significant elevation in platelet levels in BP poorly controlled female hypertensive patients as compared to BP controlled ones ($p<0.05$). However, the study showed no statistically significant variation between BP controlled and BP poorly controlled male and female hypertensive patients in their respective hemoglobin, MCV, hematocrit, MCH and MCHC levels ($p>0.05$).

In addition, as compared to the well-controlled hypertensive patients, independent samples t test showed that poorly controlled hypertensive ones had significantly higher mean levels of hemoglobin, RBC count ($p<0.05$) in all the patients. The average levels of platelets, MCH, MCHC and hematocrit were also higher, although not statistically significant ($p>0.05$), in uncontrolled patients as compared to the controlled ones.

Table 5: Levels of hematological parameters in BP controlled and BP poorly controlled male and female hypertensive patients at Debre Markos Referral Hospital, Ethiopia, October 2016 to January 2017

Variables	Males (n=55)			Females (n=45)		
	BP controlled	BP poorly controlled	p-value	BP controlled	BP poorly controlled	p-value
RBCx10[6] cells/µl	4.65±0.57	4.92±0.44	0.049*	4.48±0.47	4.63±0.44	0.288
WBCx10[3] cells/µl	6.24±1.54	7.71±2.50	0.009*	8.03±3.49	8.09±2.05	0.95
Platelets[a]	234.73±76.2	212.15±76.51	0.288	224.38±59.28	278.93±70.76	0.012*
MCV[b]	90.00±4.59	88.70±2.68	0.187	88.35±4.74	88.97±2.69	0.57
MCH[c]	31.64±2.08	31.10±1.06	0.211	30.65±2.35	31.62±1.79	0.12
MCHC[d]	34.46±1.77	35.08±0.84	0.087	34.70±1.14	35.12±13.08	0.18
Hemoglobin[e]	14.77±1.45	15.47±1.37	0.078	13.79±2.03	14.68±1.78	0.13
Hematocrit[d]	42.48±3.90	44.10±4.10	0.15	39.59±5.25	41.07±4.24	0.30

Data are expressed as Mean ±SD; *P value ≤0.05 is statistically significant. Values bearing different superscripts [a, b, c, d, e] represent units, [a]10^3 x cells/µl; [b]femtoliter; [c]picogram; [d]percent; [e]gram per deciliter.

4.5. Sociodemographic characteristics and the dependent variables

Bivariate, Pearson correlation, analyses showed that age was positively correlated with serum, LDL-C ($r = 0.274$, $p < 0.05$); HDL-C ($r = 0.310$, $p < 0.05$); TC ($r = 0.399$, $p < 0.05$), TG ($r = 0.087$, $p > 0.05$, insignificant) in the hypertensive patients. Linear regression analysis also showed that 7.5 %, 9.6 %, 7.2 %, 0.8% of the variations of serum LDL-C, HDL-C, TC, TG levels respectively are explained by age. Although all the lipid profiles tend to be higher in patients who did not perform physical activity than who did, independent samples t tests did not show any significant variation in the levels of lipid profiles in the patients. However, Chi-square test (fishers exact test) showed that patients who did not perform physical exercise had serum TG level above the cut-off value ($p<0.05$).

Independent samples t test also showed that the mean serum TC level was significantly higher ($p<0.05$) in hypertensive patients who had been drinking alcohol than who had not been drinking. In addition, abnormal lipid profiles prevailed in patients having smoking habit.

Whereas correlation analyses showed that age is positively associated with the RBC count ($r = 0.290$, $p<0.05$ and levels of hematocrit ($r=0.197$, $p< 0.05$), it is correlated with the platelet count ($r = -0.087$, $p>0.05$ and levels of WBC count ($r = -0.131$, $p> 0.05$). Also, linear regression analyses showed that 3.9 %, 8.4% of the variations in hematocrit and RBC levels respectively can be explained by age.

4.6. Anthropometric, clinical features and the dependent variables

In the hypertensive patients, there was positive association between BMI and the lipid profiles but that was not statistically significant ($p>0.05$). However, waist circumference had statistically significant positive correlation with the serum levels of TC ($p< 0.05$) and weak association with TG, LDL-C and HDL-C levels ($p>0.05$). Correlation analysis also showed that there was positive association between waist to hip ratio and TG, TC, LDL-C levels and inverse relation with HDL but the associations were weak ($p>0.05$). In the hypertensive patients, systolic blood pressure had statistically significant correlation with LDL-C ($r =0.311$, $p < 0.05$) and TG ($r =0.311$, $p < 0.05$).

One-way ANOVA with Tukey post hoc test also showed that there was a statistically significant variation in the serum TG level between the hypertensive patients who had followed the care for more than five years as compared to those who had followed the care for less than a year ($p < 0.05$).

Among the hypertensive subjects, there was a statistically significant positive correlation between systolic blood pressure and RBC count ($r =0.249$, $P =0.013$); diastolic blood pressure and RBC count ($r =0.303$, $P = 0.002$); waist circumference and WBC count ($r =0.418$, $P = 0.000$); diastolic blood pressure and platelet count ($r = 0.212$, $P=0.035$); waist to hip ratio and MCV ($r = 0.225$, $P = 0.024$); systolic blood pressure and hemoglobin ($r = 0.212$, $P = 0.034$); diastolic blood pressure and hemoglobin ($r = 0.296$, $P = 0.003$); diastolic blood pressure and hematocrit ($r = 0.229$, $P = 0.022$). On the other hand, RBC count showed a statistically significant inverse association with waist-to-hip ratio ($r =- 0.198$, $P = 0.048$) in the hypertensive patients.

5. DISCUSSION

The present study evaluated the serum lipid parameters (LDL-C, HDL-C, TC and TG) as well as hematological parameters (RBC, WBC, hematocrit, hemoglobin, Platelets, MCV, MCH, MCHC) in outpatient hypertensive subjects. A total of 100 hypertensive patients were involved in the study at Debre Markos referral hospital, Ethiopia. Significantly larger proportions of the patients were found to have elevated levels of TC, TG, LDL-C. Some hematological parameters such as hematocrit, WBC and RBC were also increased in parallel with the arterial blood pressure in the patients. Anthropometric indicators like waist circumference were also higher than their respective cut off values in the hypertensive patients. The present study discusses the findings of the lipid abnormalities and hematological parameters as well as anthropometric indices with respect to hypertension and their implication as risk factors of cardiovascular diseases.

5.1. Levels of lipid profiles in the hypertensive patients

The result of this study revealed that the average levels of serum TC and TG were found to be higher than their respective cut-off values. In addition, the mean LDL-cholesterol and HDL-cholesterol levels were significantly higher than their respective cut-off values. These higher mean levels of TC, TG and LDL-cholesterol in hypertensive patients are in agreement with the results of other related studies which are conducted in different parts of the world including Ethiopia (Forhand *et al*., 2014, Ghooshchi *et al*., 2014, Prabhanjan *et al*., 2014, Srivastava and Binawara, 2016).

A rising trend was also observed for prevalence of lipid abnormalities and serum levels of TG, TC, LDL-C, and HDL-C (decreasing serum level) with the severity of hypertension (i.e. controlled versus poorly controlled hypertensive patients indicating that they are associated with hypertension. These results are in trajectory with a study done by Nayak *et al* (Nayak *et al*., 2016).

In the present study, the results of the prevalence of different lipid profile abnormalities have been summarized as per the criteria of NCEP ATP III. Abnormally high serum level of LDL-C was the most frequently occurring serum lipid profile abnormalities among the hypertensive patients, followed by high level of TC and TG. However, low HDL-C was found to be the most infrequent lipid abnormality in the hypertensive patients. But, the abnormalities often occurred together rather than in isolation. These findings are comparable with a study done in Nigeria by Charles Osuji *et al* 2012 who reported that abnormally high serum level of TC was the most frequently occurring serum lipid profile abnormalities among newly diagnosed Nigerian hypertensive patients followed by high level of LDL-C, and low HDL-C. However, elevated TG was found to be the most infrequent lipid abnormality in their study (Osuji *et al*., 2012).

Despite the comparable results of serum TC, TG, and LDL-C with other studies done in different parts of the world, the study has shown that the serum level of HDL-C was found to be higher as compared to most of the previous studies. But, the elevated HDL-C level was in line with one study done in Nigeria (Idemudia and Ugwuja, 2008). The reason for this variation in the study area is not clear but may partially be explained that larger proportion of the patients are alcohol drinkers and it is well established that moderate alcohol intake raises HDL-C level by increasing the transport rate of apolipoproteins A-I and A-II. In addition, genetic variation may also be attributed to the changes in HDL-C levels (Elizabeth *et al*., 2000, Burger *et al*., 2004), and clinical implications of elevated HDL-C in hypercholesterolemic hypertensive patients remained unclear.

Dyslipidemia and hypertension are well recognized and frequently coexisting major cardiovascular disease risk factors (Karthikeyan *et al*., 2009). Abnormalities in serum lipid profiles play a central role in endothelial functional abnormality which is important in the pathogenesis of atherosclerosis, thrombosis, insulin resistance as well hypertension. Lipoproteins rich in triglyceride and LDL- cholesterol have been recognized to be toxic to endothelium, while HDL cholesterol may have protective role. Abnormally high serum total cholesterol levels are considered to be risk factors for developing macrovascular complications like Coronary Heart Disease (CHD), stroke and hypertension (Albuche *et al*., 2000).

5.2. Levels of hematological parameters in hypertensive patients

This study showed statistically significant elevation in WBC and RBC levels between BP controlled and BP poorly controlled male hypertensive patients whereas it showed statistically significant variation in platelet levels between BP controlled and BP poorly controlled female hypertensive patients. The average levels of hemoglobin and hematocrit were also higher, although not statistically significant, in BP poorly controlled hypertensive patients as compared to the controlled ones.

Studies have shown that white blood cell (WBC) count has been found to be associated with hypertension and its complications (Karthikeyan and Lip, 2006). Inflammation may contribute to increasing resistance of microvascular capillary, initiation of platelet aggregation, increased levels of catecholamine, and there is considerable evidence of an association between inflammation and hypertension (Bautista *et al*., 2001).

Hemoglobin is most important determinant of whole blood viscosity (Simone *et al*., 1990). Studies have shown that the concentrations of hemoglobin increased with hypertension in humans. However, only a limited number of large-population based studies have shown a link between hemoglobin concentration and blood pressure. In another study conducted among unselected public employees who did not receive any medication, hemoglobin concentration was significantly associated with hypertension (Kawamoto *et al.*, 2012, Shimizu *et al*., 2014).

In a recent study involving a large cohort of blood donors who were relatively healthy, hemoglobin concentrations were positively associated with both systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Atsma *et al*., 2012, Shimizu *et al*., 2016). Researchers also reported that three erythrocyte parameters (RBC, hemoglobin, and hematocrit) were found to be associated with hypertension in their cohort study. Hematocrit, the proportion of blood volume occupied by red blood cells, determines blood viscosity, regulates peripheral vascular resistance (PVR) and therefore, in principle, blood pressure (Wu *et al*., 2013, Shimizu *et al*., 2016). Therefore, this study generally is concordant to numerous studies conducted in hypertensive subjects.

5.3. Sociodemographic characteristics and the dependent variables

The majority of the hypertensive patients were found within in the age group of 40-59. This is in line with the previous studies done both in developed and developing countries which consistently reported that age is associated with hypertension (Idemudia and Ugwuja, 2008, Forhand *et al*., 2014). In addition, the present study revealed that age was positively correlated with serum, LDL-C ($r = 0.274$, $p < 0.05$); HDL-C ($r = 0.310$, $p < 0.05$); TC ($r = 0.399$, $p < 0.05$), TG ($r = 0.087$, $p > 0.05$, insignificant) in the hypertensive patients. This finding is in corroboration with the previous studies (Osuji *et al*., 2012, Prabhanjan *et al*., 2014). As we age, there is a natural tendency for the blood pressure to rise which could be because of an increase in stiffness of the arteries in the vasculature as well as endothelial atherosclerotic changes.

The results of epidemiological studies have revealed the relation of age with arterial stiffness in patients with hypertension; as age advances, so do the prevalence of hypertension and arterial stiffness (Ferreira *et al*., 2012, Alghatrif *et al*., 2013). Hypertension is usually related with other cardiovascular risk factors such as dyslipidemia (abnormal levels of lipids and lipoproteins in the blood), diabetes, and obesity. The presence of these cardiovascular risk factors and the resulting endothelial dysfunction may play a role in the pathophysiology of hypertension (Oparil *et al*., 2003).

The present study also showed that the mean serum TC level was higher in hypertensive patients who had been drinking alcohol than who had not been drinking. In addition, abnormal lipid profiles prevailed in patients having smoking habit, which is in line with a study done in Greece (Mammas *et al*., 2003).

5.4. Anthropometric, clinical features and the dependent variables

The present study showed that most of the hypertensive patients (62%) had poorly controlled blood pressure: only 38% of patients had well controlled blood pressure. Although the study did not assess reasons for such high proportion of poorly controlled hypertensive patients, it could possibly and partly be attributed to noncompliance to antihypertensive drugs, poor follow-up in the hospital, lack of adequate health education and counseling related with hypertension and its precipitating risk factors, and financial constraints for antihypertensive drugs and care.

Anthropometric indicators are related with different pathological conditions. Although BMI is widely used indicator to reflect obesity generally, it fails to account the proportion of weight related to muscle mass or regional distribution of excess fat in the body, both of which influence the health risks related with obesity. Individuals having same BMI may significantly vary in their abdominal fat distribution or mass (Chehrei *et al*., 2007). For these reasons, a measure of obesity that takes into account the increased risk of obesity related illnesses because of the accumulation of abdominal fat is desirable. As a result, there is a new tendency to use waist circumference and waist to hip ratio.

This study tried to investigate the associations of some anthropometric indices (BMI, WC, and waist-to-hip ratio) and lipid abnormalities in hypertensive patients in the study area. Concordant to the previous studies (Lorenzo *et al*., 2016), the result of this study showed that there is correlation between the anthropometric indicators and lipid abnormalities.

There was positive association between BMI and the lipid profiles. In addition, waist circumference had significant positive association with the serum levels of TC and weak association with TG, LDL-C and HDL-C levels. Correlation analysis also showed that there was positive association between waist to hip ratio and TG, TC, LDL-C levels and inverse relation with HDL. TC level, among lipid profiles, showed the closest relationship with WC and waist-to-hip ratio. As regression analysis of the study showed WC and waist-to-hip ratio can better predict lipid abnormalities in the hypertensive patients.

An increased waist circumference is most likely associated with elevated risk factors because of its relation with visceral fat accumulation. The mechanism may involve excess exposure of the liver to fatty acids and release of detrimental adipocytokines and lower levels of beneficial adipocytokines. These have multiple detrimental effects, including proinflammatory damage, altered signalling pathways and reactive oxygen species production, on beta cells and other tissues resulting in disease states like hypertension and diabetes (Dalton *et al*., 2003).

In addition, the accumulation of visceral fat may bring about an increase in sympathetic over activity which is associated with insulin resistance and hence increasing the activity of the renin-angiotensin-aldosterone system as visceral adipocytes increase angiotensinogen secretion as compared to the subcutaneous fat (Lee *et al*., 2006). Mechanical effect could also be exerted by the accumulation of visceral fat resulting in renal compression and promoting a rise in arterial blood pressure (Hall *et al*., 2003).

In the hypertensive patients, systolic blood pressure had statistically significant correlation with serum LDL-C and TG levels which tended to rise as the duration of hypertension advances. Plethora of studies such as a study conducted in Europe (Borghi *et al*., 2016), another study carried out in India (Divya and Ashok, 2016), in Nigeria (Osuji *et al*., 2012), as well as a study conducted in Ethiopia (Prabhanjan *et al*., 2014) are in trajectory with the present study.

Hypertension and lipid abnormalities are well known to frequently coexist and synergize to be risk factors for CVD. The coexistence of increased blood pressure and lipid abnormalities has many clinical implications. Because hypertension and lipid abnormalities synergize to be risk factors for CVD, both of them should cautiously be intervened. Central obesity and consequent insulin resistance which are underlying factors that play major roles in the pathogenesis of both hypertension and dyslipidemia may link the association. Lipid abnormalities, characteristic of metabolic syndrome, was found to predict hypertension and it had also been shown in cohort studies that dyslipidemia in apparently healthy individuals leads to hypertension (Halperin *et al*., 2006, Laaksonen *et al*., 2008).

The present study also revealed that blood pressure had statistically significant positive correlation with RBC count, hemoglobin, hematocrit and platelet levels. Although this finding is in corroboration with some of the earlier studies(Al-Muhana *et al*., 2006, Atsma *et al*., 2012), it is unlike the finding of Divya and Ashok who reported that hemoglobin and hematocrit showed a negative correlation with systolic blood pressure among hypertensive patients whom they studied (Divya and Ashok, 2016).

The present study also showed that waist circumference and WBC count had significant association. Similar finding had been reported in studies conducted in South Korea (kim and Park, 2008) as well as Iran (Farhangi et al., 2013). On the other hand, RBC count showed a statistically significant inverse association with waist-to-hip ratio in the hypertensive patients. As discussed above, waist circumference is related with visceral fat accumulation which leads to release of detrimental proinflamatory cytokines that can increase the WBC count.

6. CONCLUSIONS

The study concluded that the hypertensive patients in the study area have high prevalence of lipid profile abnormalities and poorly controlled blood pressure. Some hematological parameters like RBC count, WBC count were also increased in hypertensive patients as a factor of their blood pressure increases. Significantly higher proportions of the hypertensive patients were overweight and obese which seems to contradict the claim that overweight in Ethiopian population is less prevalent. TC level, among lipid profiles, showed the closest relationship with WC and waist-to-hip ratio. WC and waist-to-hip ratio can better predict lipid abnormalities in the hypertensive patients.

7. STRENGTHS AND LIMITATIONS OF THE STUDY

The study can express its strength that it includes several demographic, clinical and anthropometric parameters claimed to be associated with the variables under study. In addition, the anthropometric indicators are measured directly than by self-report.

Despite the aforementioned strengths, this study has several weaknesses. As the study was conducted in only one referral hospital and the sample size was small, it may be difficult to represent the whole hypertensive patients in the population. In addition, the study could not compare the effects of lipid and hematological profiles variations in dietary habits. Lack of ample previous study findings limited the comparison of these study findings with other findings in similar hospitals in Ethiopia. Finally, being a cross-sectional study by design it cannot observe prospectively and thus cannot associate causal relationships between the factors under study.

8. RECOMMENDATIONS

The following recommendations are suggested to further investigate and evaluate lipid profiles and hematological parameters in hypertensive patients.

- Further studies could be conducted with larger sample size and incorporating more sample hospitals as well as using more robust study designs such as case control and cohort to establish the causality of the association between hypertension and lipid abnormalities and their implications on the management of hypertensive patients.
- Nutritional factors and dietary habits should be assessed in further studies.
- There should be better public education and care as well as clinical care of the hypertensive patients for better control of blood pressure and lipid abnormalities as well as overweight and obesity.
- Further molecular studies could also be directed as to investigate the genetic basis of the such high prevalence of lipid abnormalities in the hypertensive patients.
- There should be timely evaluation of lipid profile of the patients and prescription of drugs whenever indicated so as to prevent CVD complications.

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10.ANNEXES

10.1. Annex 1: Information sheet (English Version)

Research Project: Evaluation of lipid profiles and hematological parameters in hypertensive patients at Debre Markos Referral Hospital

Sponsoring organization: Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University

Principal Investigator: Alemu Gebrie (Bpharm, MSc in biochemistry candidate)

Advisors: Gnana Sekaran (PhD), Menakath Menon (PhD)

Introduction

Dear the participants you are kindly requested to take part in this research project as a study participant voluntarily. Read the information provided in this sheet carefully and then respond freely and voluntarily to what the investigator interviews you.

Objective of the research project

This information sheet is prepared by the investigator and the advisors at AAU for a project with the objective of evaluation of lipid profiles and hematological parameters among hypertensive participants.

Procedure

If you agree to take part in the study, the investigator or a health worker will give you verbal and/or written information about the study and you will be given the consent from to sign, the physician or health professional will ask you some questions about your general health and perform a complete medical examination and assess whether you qualify to participate in the study. If you are fit for the study about 5 ml of blood samples will also be collected for only the laboratory examination of complete blood count, HDL, LDL-C, total cholesterol, triglycerides and face to face interview for additional questions.

Discomforts and risks and benefits from participation

The degree of discomfort you may encounter in giving the sample is no more than when one does in his/her routine examination. But, there could be cases in which minor pain and change in color of your skin following the blood drawing occur transiently. The blood will be withdrawn by licensed health care professionals in the hospital and appropriate care will also be taken. You will not be provided with any direct incentives for your participation in the research. But the cost for general medical examination will be covered by the project. In addition, based on the results obtained from the research you will be cared accordingly or the results may serve you as a baseline data. In addition, the result of the study will be beneficial for the better prevention and care of hypertensive patients than before. Hence, you are indirectly benefiting other patients and the society in this aspect.

Confidentiality

All pieces of information about the patients will be kept confidential. Log books used in the laboratory will have no names but codes. The information sheet that links the coded number to patient name will be locked inside a box and it will not be revealed to anyone except your physician and the principal investigator. You have full right to withdraw from participating in this study at any time before and after consent even without explaining the reason. Your decision will not affect your right to get health service you are supposed to get otherwise.

Contact information: If you have any questions contact: Alemu Gebrie: 0936633883

10.2. Annex 2: Informed consent (English version)

Department of medical Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa University, Consent form for the participation of the study participants in the research project

Name of the study participant ………………………

Code number…......................

I have clearly been informed about the research project that it aims to evaluate and correlate serum lipid panels and hematological parameters among hypertensive patients. The objectives of the research project have clearly been explained to me and I have been told that the results obtained from me will help me as well as the community for better management of the disease. I had been also informed about the confidentiality of this research project. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_______________________________________ hereby give my consent for providing the requested information and blood sample as the doctors find best for me.

Signature: __________________________________ Date_________________________

10.3. Annex 3: Questionnaire (English version)

Dear respondents, you are kindly requested to give correct information accordingly. Thank you for your time and participation.

I. Personal sociodemographic, anthropometric and clinical information

Card no.________________

1. Age (in years) _____________
2. Sex: Male ☐ Female ☐
3. Educational status: Illiterate ☐

   Up to Secondary school ☐

   University degree ☐

4. Marital status: Single ☐

   Married ☐

   Divorced ☐

   Widowed ☐

5. Regular physical activity: Yes ☐ No ☐

6. Residential area Urban ☐ Rural ☐

7. Height (m) _________________

8. Weight (in Kg) _______________

9. Body Mass Index (kg/ m^2) ____________________

10. Waist circumference (cm) ___________

11. Hip circumference (cm) ________________

12. Waist-to-hip ratio__________________

13. Alcohol consumption: Yes ☐ No ☐

14. Smoking: Yes ☐ No ☐

15. Family history of hypertension Yes ☐ No ☐

16. Duration of hypertension: < 1 year ☐

1-5 years ☐

>5 years ☐

10.4. Annex 4: Information sheet (Amharic version)

የተሳታፊዎች የፈቃደኝነትና መተማመኛ መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ትምሕርት ክፍል፡

ጥናቱን ስፖንሰር ያደረገዉ ተቋም አዲስ አበባ ዩኒቨርሲቲ ጤና ሣይንስ ኮሌጅ ነው።

መረጃ መስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ት/ክፍል ሁለተኛ ዲግሪ ተማሪ የመመረቂያ ጥናት ጽሁፍ ላይ እዲሳተፉ ተጋብዘዋል። እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉንም ሃሳብ ይጠይቁ።

Evaluation of lipid profiles and hematological parameters in hypertensive patients at Debre Markos Referral Hospital የጥናቱ ርእስ ሲሆን አላማውም የደም ግፊት ያለባቸው ታካሚዎች በደማቸው ውስጥ ያለውን የቅባት መጠንና የደም ህዋሶች እንዲሁም ሌሎች ከደም ግፊት ጋር ግንኙነት ያላቸውን ነገሮች መጠንና ሁኔታ መለካት ነው። የጥናቱ ውጤት ለታካሚው ብሎም ለሌላው ማህበረሰብ የሚጠቅምና የተሻለ የጤና እንክብካቤ እንዲኖር የሚያደርግ ነው።እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቃሚና ምቹ ሆነው ተመርጠዋል። የእርስዎ በዚህ ጥናት ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ።

በጥናቱ ከተሳተፉ ለናሙና ይሆን ዘንድ 5ሚሊ ሊትር ያህል ደም በሆስፒታሉ ጤና ባለሙያዎች የሚሰጡ ሲሆን የደም ናሙናውን በሚሰጡበትም ሰአት ሁልጊዜ ለምርመራ ከሚሰጡበት የተለየ ህመምና አለመመቸት የለውም ለምናልባት ቢኖር ተገቢውን የጤና እንክብካቤ የሚያገኙ ይሆናል። በዚህ ጥናት

ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዎ ማንኛዉም አገልግሎት ላይ ተጽዕኖ የለውም። በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅበዎታል፡

ግልጽ ያልሆነልዎ ጥያቄ ካለ

ሞባል፡ 0936633883 አለሙ ገብሬ

10.5. Annex 5: Informed consent (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር -----------------------------

የተሳታፊዉ ስም ------------------------------

እኔ ስሜ ከላይ የተገለጸዉ ግለሰብ የተፈለኩት በዚህ ጥናት እንድሳተፍ ሲሆን የደም ግፊት ያለባቸው ታካሚዎች በደማቸው ውስጥ ያለውን የቅባት መጠንና የደም ህዋሶች እንዲሁም ሌሎች ከደም ግፊት ጋር ግንኙነት ያላቸውን ነገሮች መጠንና ሁኔታ መለካት የሚለው ጥናት አላማና ጥቅም ተገልፆልኛል። ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው። በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደማይባክን እንደሚያዝም ተነግሮኛል፡ ፡ በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል።

ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለሁ። በተጨማሪም የምሰጠዉ የደም ናሙና ለCholesterol,Triglycerides,HDL-C LDL-C እና complete blood count ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ። ማንኛዉንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ።

በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ለሀኪሜ እንደሚሰጥልኝ እና ዉጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል። በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤአለሁ። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ።

እኔ____የተባልኩት ግለሰብ ይህን ሁሉ በማገናዘብ በምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ።

ፊርማ ቀን

ተሳታፊ ________________________ ____________________

10.6. Annex 6: Questionnaire (Amharic version)

መጠይቅ

ውድ ተሳታፊ ቀጥሎ ያለውን መጠይቅ ለመሙላት ስለተባበሩን እናመሰግናለን፡፡

ካርድ ቁጥር_______________

1. እድሜ (በአመት) _____________

2. ጾታ: ወንድ ☐ ሴት ☐

3. የትምሕርት ደረጃ: ማንበብና መጻፍ የማይችል ☐

እስከ ሁለተኛ ደረጃ ☐

የዩኒቨረሲቲ ዲግሪ ☐

4. የትዳር ሁኔታ: ያላገባ ☐

ያገባ ☐

የፈታ ☐

የሞተበት ☐

5. መደበኛ የአካል ብካት እንቅስካሴ: አዎ ☐ አላደርግም ☐

6. መኖሪያ ቦታ፡ ከተማ ☐ ገጠር ☐

7. የሰዉነት ክብደት ልኬት (ኪ.ግ. / ሜ^2) _________________

8. ክብደት (በኪ.ግ.) _______________

9. ቁመት (በሜትር)____________________

10. የወገብ ዙሪያ (በሴ.ሜ.)___________

11. የዳሌ ዙሪያ (በሴ.ሜ.) _______________________

12. ወገብ-ለ-ዳሌ ንጽጽር____________________________

13. አልኮል ይጠጣሉ: አወ ☐ አልጠጣም ☐

14. ሲጋራ ያጨሳሉ: አወ ☐ አላጨስም ☐

15. የደም ግፊት ያለበት ዘመድ አለዎት፡ አወ ☐ የለኝም ☐

16. የደም ግፊት ከተመረመሩ ስንት ጊዜ ሆነዎት: < 1 አመት ☐

1-5 አመት ☐

> 5 አመት ☐