ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL BIOCHEMISTRY

ASSESSMENT OF PLASMA D-DIMER LEVEL AND ITS CORRELATION WITH DISEASE SEVERITY AMONG HYPERTENSIVE PATIENTS

By:

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DECLARATION FORM

I, the undersigned, declare that this thesis report entitled "Assessment of Plasma D-Dimer level and its Correlation with Disease Severity among Hypertensive Patients" was my own original work and it has not been proposed and presented in other universities, colleges or other institutions for similar purpose, and that all sources of materials used for the research have been properly and suitably acknowledged.

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Signature Date

This is to certify that this master's thesis was prepared by Endeshaw Chekol, and submitted to the Department of Biochemistry in requirements for partial fulfillment of degree of Master's science in Medical Biochemistry, with regulation of university and meet acceptance standards with respect to originality and quality.

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ABBREVIATIN AND ACRONYMS

ACC…… American College of Cardiology

AHA ……American Heart Association

BP ..……..Blood Pressure

BMI……..Body Mass Index

CHD..……Coronary Heart Disease

CVD …… Cardiovascular disease

DALYs……. Disability-Adjusted Life Years

DBP…..……. Diastolic Blood Pressure

D-DI ……..D-Dimer

DIC ……Disseminated Intravascular Coagulation

DVT ……...Deep Venous Thrombosis

ESRD ……End-Stage Renal Disease

FDP ……Fibrin degradation Product

HTN……Hypertension

JNC 7 …….Seventh Report of the Joint National Committee

NCD ……..Non-communicable diseases

NHANES …….National Health and Nutrition Examination Survey

PAD……..Peripheral Arterial Disease

PAI-1…….....Plasminogen Activator Inhibitor 1

ROC ……Receiver Operating Curve

SBP ……..Systolic Blood Pressure

SPSS ……Statistical Package for the Social Sciences

TOD ……Target Organ Damage

tPA ……tissue Plasminogen Activator

VTE ……..Venous Thromboembolism

vWF …….von Willbrand Factor

WHO..……..World Health Organization

Y12HMC ……..Yikatit 12 Hospital Medical College

ABSTRACT

Introduction: Hypertension is a single most important risk factor for cardiovascular disease, and the growing public health issue in developing countries. It has been reported that inappropriate acute thrombus formation (hypercoagulability) is the pathophysiological substrate underlying increased risk and severity of target organ damage of hypertension, such as acute coronary syndrome and stroke that can cause significant morbidity and mortality. Plasma D-dimer level has been reported to be a good biochemical marker of thrombosis.

Objectives: The objective of this study was to assess plasma D-dimer level and its correlation with disease severity among hypertensive patients at Yikatit 12 Hospital Medical College.

Methods: A hospital based comparative cross-sectional study was conducted at Y12HMC. Hundred participants (60 hypertensives and 40 controls) were recruited in the study. Data were collected using structured questionnaire through face to face interview, reviewing medical records and direct measurement of variables. The data was analyzed using SPSS version 25.0 and categorical variables were described by frequency and percentage while continuous variables by mean and standard deviation. The association and correlation between variables were determined using correlation coefficients, regression analysis, and also using different parametric and non-parametric tests accordingly.

Results: Among study participants, female to male ratio was 1.4 and the mean age was 52 year with a range of 20 to 80 years. Among hypertensives,30(50.0%) were in stage 1 while 14(23.3%) and 16(26.7%) were in stage 2 and severe hypertension respectively. We observed higher D-dimer levels among hypertensives when compared with the healthy controls ($p<0.001$). The plasma D-dimer levels were found to be increased significantly with the severity of hypertension ($p<0.001$). D-dimer found to have a diagnostic power of 86.9% in differentiating complicated from uncomplicated hypertension at 0.83mg FEU/l cut-off value.

Conclusion and recommendations: This study suggests that D-dimer level was higher in hypertensives than control groups and it was also increasing significantly with the severity of hypertension suggesting hypercoagulability plays a role in the pathogenesis of cardiovascular disorders and thromboembolic complications of hypertension. But further studies need be done on larger scale and using more robust study designs such as case control and cohort to establish the causality of the association between severity of hypertension and D-dimer level.

Key words: Hypertension, D-dimer, Yikatit 12 Hospital Medical College

1. INTRODUCTION

1.1 BACKGROUND

Non-communicable diseases (NCDs) are by far the leading cause of death worldwide. In 2016, they were responsible for 71% (41 million) of the 57 million deaths which occurred globally. The major NCDs responsible for these deaths included cardiovascular diseases (CVD) (17.9 million deaths, accounting for 44% of all NCD deaths and 31% of all global deaths)(WHO, 2018). Cardiovascular disease (CVD) is the leading (48 %) cause of NCD death in developed countries (Thom, 1992), and there is growing evidence that a similar epidemic is highly prevailing in the developing world including Ethiopia (Kibret and Mesfin, 2015;Asresahegn *et al.*, 2017).

Hypertension has for long been globally recognized as the most prevalent CVD, which is a medical condition with a characteristic feature of chronically elevated state of systemic blood pressure (BP) (Chobanian *et al.*, 2003). It is the most important modifiable cardiovascular risk factor that is closely associated with lethal complications like coronary heart disease (CHD), stroke, congestive heart failure (CHF), end-stage renal disease (ESRD) and peripheral vascular disease (PAD) (Chobanian *et al.*, 2003).

According to the 2017 American College of Cardiology/American Heart Association (ACC/AHA) guideline, BP thresholds for defining hypertension has been updated for the initiation of and goals of pharmacologic treatment of hypertension based on a pragmatic interpretation of BP-related CVD risk and benefit of BP reduction in clinical trials (Carey and Whelton, 2018). This guideline which recommends lower thresholds than prior guidelines, defines hypertension as any one of the following in at least in two or more seated BP readings during each of two or more outpatient visits; any SBP measurement of 130 mm Hg or higher or any DBP measurement of 80 mm Hg or higher, or reported current use of antihypertensive medication. Accordingly, BP is classified as normal, elevated BP (instead of prehypertension), stage1 hypertension and stage 2 hypertension (**Table 1**).

Table 1:Blood pressure classification (Carey and Whelton, 2018).

Category	Systolic BP (mmHg)	Diastolic BP (mmHg)
Normal	<120	<80
Elevated	120-129	<80
Stage 1 hypertension	130-139	80-89
Stage 2 hypertension	≥140	≥90
Hypertensive crisis	Over 180	Over 120

The severity of hypertension and hence the cardiovascular risk or target organ damage (TOD) increases as one goes from normal BP to stage 2 hypertension and hypertensive crisis (Muntner *et al.,* 2017). Similarly, though there is no obvious level of BP that defines hypertension from an epidemiologic perspective, there is a continuous incremental risk of CVD, stroke, and renal disease across levels of both SBP and DBP in adults (Chobanian *et al.*, 2003).

Severe clinical syndromes which occur as complications of untreated or inadequately treated hypertension and are a frequent reason patient present to health care facilities are called hypertensive crisis (Gifford, 1991;Elliott, 2006). This condition categorized as hypertensive urgency and emergency based on evidence of acute TOD such as CVD and cerebrovascular disorder. Hypertensive emergency is defined as severe hypertension accompanied by acute end organ dysfunction; whereas, hypertensive urgency is defined as severely elevated BP (a SBP≥ 180 or DBP≥ 120) without acute end-organ damage (Gifford, 1991).

White coat hypertension (WCH), isolated clinic hypertension, is defined as the documentation of increased BP (> 140/90 mmHg) in several visits to the clinic, while 24-hr ambulatory blood pressure monitoring (ABPM) levels are < 125/80 mmHg (Chobanian *et al.*, 2003). WCH is a consequence of stimulation of the autonomic and central nervous systems during situations of stress, anxiety or excitement and the prevalence of which varies from 15% to 35%.(Pickering *et al.*, 1999;Jepson, 2011).

Only about 5-10 % hypertensives have a specific identified underlying disorder causing the elevation of BP called “secondary hypertension” as is mostly secondary to renal disease or

less commonly to renal artery stenosis (called renovascular hypertension), endocrine abnormalities, vascular malformations, or neurogenic disorders (Mitchell, 2006). The rest majority (90-95%) of hypertension, however, has no known exact cause and diagnosed as having "primary or essential hypertension" (Beevers *et al.*, 2001;Tomson, 2005).

The frequent and the only sign of essential hypertension is elevated BP and it is recognized as the "silent killer" because it typically has no warning signs or symptoms, and many people even do not know they have it (James *et al.*, 2014).

Many recent guidelines on hypertension diagnosis and management 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 (WHO, 2013).

D-dimer, which has an estimated weight of 195 kDa, is a plasma plasmin derived degradation product of cross-linked fibrin (Olson, 2015). It was first identified and isolated in the 1970s (Gaffney, 1973). D-Dimer is a marker of increased fibrin turnover and ongoing intravascular fibrin formation, and it is a widely used indicator for the exclusion of venous thromboembolism (VTE) with highly negative predictive value(Wells *et al.*, 2003). Similarly, elevations of D-dimer are associated with increased risk of future TOD like myocardial infarction, stroke, and PAD (Lowe, 2005).

1.2 LITRATURE REVIEW

1.2.1 Epidemiology of Hypertension

Hypertension is an overwhelming global challenge, which is the 4th contributor to premature deaths in developed countries and the 7th in developing countries (Dreisbach, 2013). Globally, nearly one billion people have hypertension and it ranks third in disability-adjusted life-years (DALYs) (Kearney, 2005). The prevalence of raised BP in adults older than 25 years of age was about 40 % in 2008 and contributed to 12.8 % of the total deaths in the world (WHO, 2011, 2013). The World Health Organization (WHO) also estimated that around 62 % of CVDs and 49 % of ischemic heart diseases are attributable to high BP (WHO, 2013).

In US, based on results of NHANES, approximately 30% (age adjusted prevalence) of adults, or at least 65 million individuals, have hypertension. The age-adjusted prevalence of hypertension is slightly higher in men than in women (Nwankwo *et al.*, 2013). According to the report from 2017 ACC/AHA Guideline, the absolute burden of hypertension is consistently increased, from 87.0 million in 1999–2000 to 108.2 million in 2015–2016 (Ioannidis, 2018).

More recently, developing countries are facing what is known as "double burden of diseases" that involves communicable diseases and NCD (including hypertension) responsible for the highest burden of morbidity and mortality in Africa (Bygbjerg, 2012). The prevalence of hypertension has been increasing in developing countries including Ethiopia. Though there is paucity of data about prevalence of hypertension at national level in Ethiopia, few small-scale studies indicate that the disease has become a significant public health problem in Ethiopia (Awoke *et al.*, 2012;Asresahegn *et al.*, 2017). A meta-analysis intended to summarize the results of smaller region based studies to provide an estimate of national level hypertension prevalence showed that the prevalence of hypertension among Ethiopian population was 19.6 % (95 % CI: 13.7 %, 25.5 %)(Kibret and Mesfin, 2015). The same subgroup analysis also indicates that though the prevalence of hypertension among males (20.6 %) and females (19.2 %) was nearly the same, it was higher in the urban population (23.7 %) than rural and urban combined (14.7 %). This is supported by a community based cross sectional study conducted in Gondar, Northwest Ethiopia where the prevalence of hypertension is 28.3% (Awoke *et al.*, 2012).

1.2.2 Risk factors and Pathophysiology of Hypertension

The complex interplay between modifiable and non-modifiable factors or conditions, including both environmental and genetic factors, are reported to play a role in the development of primary hypertension. These factors that have been intensively studied to contribute to hypertension development and even to CVD are salt intake, low dietary intakes of calcium and potassium, obesity, insulin resistance, dyslipidemia, alcohol consumption, psychosocial stress, low levels of physical activity, smoking, genetics , low birth weight and intrauterine nutrition, and neurovascular anomalies (Tomson, 2005).

A number of physiological mechanisms such as renin-angiotensin-aldosterone system (RAAS) and autonomous nervous system are involved in the maintenance of normal BP and their derangement may play a part in the development of essential hypertension. Maintenance of a normal BP is dependent on the balance between the cardiac output which is determined by stroke volume and heart rate, and the peripheral vascular resistance determined by small arteries and arterioles (Chobanian *et al.*, 2003). But in the presence of risk factors, there is derangement of the normal physiological BP control mechanisms, which include activation of RAAS, sympathetic nervous system, and vascular mechanisms that all have the potential to increase arterial pressure out of control by increasing cardiac output and peripheral vascular resistance (Beevers *et al.*, 2001).

1.2.3 Pathological Consequence of Hypertension

A. Target organ damage of Hypertension

In long-term, hypertension leads to target organ damage (TOD) or thromboembolic complications associated with heart, brain, kidney and peripheral arteries, resulting increased morbidity and mortality (MacMahon, 1990;Chobanian *et al.*, 2003;Foe¨x and Sear, 2004).

In human patients a graded relationship exists between increasing BP and cardiovascular morbidity and mortality (Jepson, 2011). Heart disease, which is the most common cause of death in hypertensive patients, is the result of structural and functional adaptations and leading to left ventricular hypertrophy, congestive heart failure (CHF), coronary heart disease (CHD), stroke, and cardiac arrhythmias. In the Framingham Heart Study, 30-year follow-up data revealed that the incidence of heart disease was proportional to the level of SBP (Poli, 2000).

Similarly, a strong association between SBP and all CVD was evident; the bulk of CVD incidence resulted from CHD events (Lowe, 2005).

Chobanian *et al* (2003) report shows that stroke is the second most frequent cause of death following CHD in the world; it accounts for 5 million deaths each year, with an additional 15 million persons having nonfatal strokes (Chobanian *et al.*, 2003). Elevated BP is the strongest risk factor for stroke. Approximately 85% of strokes are due to infarction, and the remainder are due to either intracerebral or subarachnoid hemorrhage. The incidence of stroke rises progressively with increasing BP levels, particularly SBP in individuals >65 years (Goldstein *et al.*, 2006).

The kidney is both a target and a cause of hypertension. Primary renal disease is the most common etiology of secondary hypertension. Mechanisms of kidney-related hypertension include a diminished capacity to excrete sodium, excessive renin secretion in relation to volume status, and sympathetic nervous system overactivity. Conversely, hypertension is a risk factor for renal injury and End Stage Renal Disease (ESRD)(Chobanian *et al.*, 2003;Malani, 2012).

Recent reviews also indicate that in addition to contributing to the pathogenesis of hypertension, blood vessels are a target organ for atherosclerotic disease secondary to long-standing elevated BP. In hypertensive patients, vascular disease is a major contributor to stroke, heart disease, and renal failure. Further, hypertensive patients with arterial disease of the lower extremities are at increased risk for future cardiovascular disease and ischemia (Malani, 2012;Fowkes *et al.*, 2017).

B. Hypercoagulability and Hypertension

Hypertension reported to increases the risk and severity of TOD, not only by clustering with other CVD risk factors such as hypercholesterolemia, hypertriglyceridemia, obesity, and insulin resistance but also independently (Lind and Lithell, 1993;Lip *et al.*, 1997).

Despite many efforts, the precise pathophysiological mechanisms by which elevations in arterial BP may promote TOD remains poorly understood (Lip and Blann, 2000). But, the hemostatic system may play an important role as has been recognized in the pathogenesis of

atherothrombotic events that involved in the TOD and long-term prognosis of hypertension (Rocha, 1994;Alfonso, 1997).

A comparative cross sectional study has shown that hypertensive patients tended to show a unbalanced fibrinolytic system and tendency towards a hypercoagulability and a more frequent thrombotic complications as compared to normotensive subjects, suggesting that impaired fibrinolysis plays a role in the pathogenesis of CVDs and complications of hypertension (Armas-Herna´ndez *et al.,* 2007). It has become evident that the prognostic value of rheological and hemostatic factors are predictors of cardiovascular events and raises the possibility that they are not merely markers or consequences of atherothrombotic disease, but may also contribute to its pathogenesis (Lee, 1997a).

Accumulating clinical and laboratory evidence suggests that hypertension per se may confer a prothrombotic or hypercoagulable state by accelerating development of atherosclerosis and increasing shear stress leading to plaque rupture and thrombosis (Lee, 1997b). This may explain in part why, though the arterial tree is exposed to increased BP in hypertensive patients, paradoxically, the major complications of hypertension like ischemic stroke and myocardial infarction are mainly thrombotic rather than hemorrhagic, known as the Birmingham paradox *(*Beevers *et al., 2001).* In accordance with Virchow's triad, patients with hypertension demonstrate abnormalities of blood vessel (endothelial dysfunction), blood constituents (abnormal levels of clotting factors, platelet activation and fibrinolysis) and blood flow (rheology and flow reserve) (**Figure 1**) suggesting that hypertension confers a prothrombotic state (Lee, 1997a;Lip and Li-Saw-Hee, 1998).

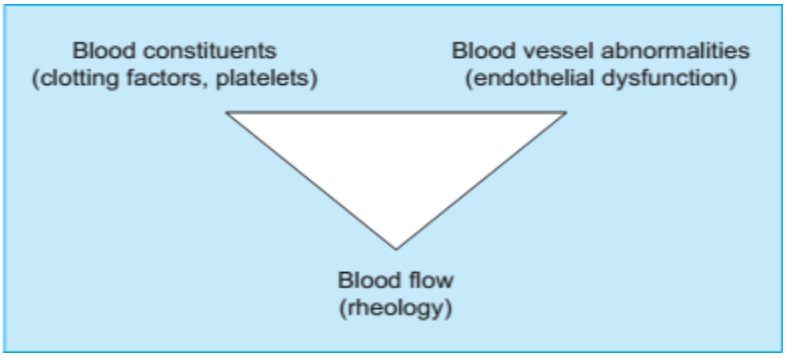

Figure 1:: Virchow's triad and prothrombotic state in hypertension (Beevers et al., 2001)

1.2.4 Hemostatic abnormalities and Hypertension

Hemostasis is a dynamic multistep physiological phenomenon that acts to coordinate the delicate balance between bleeding and clot formation by collaborating the activity of the blood vessel, platelet, and plasma proteins (Versteeg, 2013;Cimmino *et al.*, 2017). This thrombohaemmorhagic balance is maintained in the body by complicated interactions between coagulation and the fibrinolytic system as well as platelets and vessel wall.

Hypertension is characterized by hemostatic imbalance between coagulation and fibrinolysis that results a hypercoagulable state and thought to contribute for fatal cardiovascular complication *(Lip and Li-Saw-Hee, 1998).* Plethora of studies have examined that essential hypertension is associated with abnormalities in hemostatic balance caused by rheological, haemostatic, endothelial and platelet abnormalities that may act synergistically to increase the risk of thrombogenesis and atherosclerosis and thereby to the thrombotic complications in hypertensive patients (Wall, 1995;Lip *et al.*, 1997;Dell'Omo *et al.*, 2003). Several studies have demonstrated that elevated levels of fibrinogen and soluble P-selectin (a marker of platelet activation), plasminogen activator inhibitor type 1 (PAI-1) (an index of fibrinolysis), fibrin D-dimer (an index of thrombogenesis) and, abnormal hemorheology (high plasma levels of fibrinogen and plasma viscosity) in hypertensives compared to healthy normal controls (Lip *et al.*, 1997;Beevers *et al.*, 2001;Li-Saw-Hee *et al.*, 2001). Many studies demonstrate that Von Willebrand factor (vWF), a marker of endothelial function, is abnormally high in patients with hypertension, and reduced with good BP control, points to possible endothelial perturbation in hypertensive patients (Blann *et al.*, 1993;Thompson *et al.*, 1995;Zhang, 2003).

A. Coagulation profile abnormalities in Hypertension

Blood coagulation is the process of changing the blood from a liquid to a solid, through extrinsic and intrinsic pathways, by converting fibrinogen to insoluble fibrin or blood clot which is essential to prevent bleeding in the event of vascular injury (Lillicrap *et al.*, 2009;Riley *et al.*, 2016).

One of the main pathophysiological aspects of hypertension complication is derangements in coagulation profile. This is supported from the observation that satisfactory BP reduction with non-drug intervention and with various classes of antihypertensive drugs does not lead to an equal reduction in heart attacks and strokes may be due in part to unfavorable effects on the

hypercoagulable state in hypertension (Lip and Li-Saw-Hee, 1998). Measurement of plasma levels of fibrinogen, which is a clotting factor (Riley et al., 2016), prothrombin fragment 1+2, which is released when factor Xa converts prothrombin to thrombin(Mannucci and Giangrande 1992), and fibrin D-dimer, the principal breakdown fragment of fibrin (Riley et al., 2016), can be used to estimate the state of activation of the coagulation system. In patients with hypertension, plasma levels of fibrinogen, prothrombin fragment 1+2 and D-dimer are increased (Catena *et al.,* 2000), and antithrombin III activity is decreased (Vaziri *et al.*, 1994) compared with normal controls, suggesting the coagulation system is activated in these patients.

B. Fibrinolytic abnormalities in Hypertension

Abnormality in fibrinolytic pathways, in most occasion hypo-function of fibrinolysis, has been reported in patients with hypertension (Alfonso, 1997;Gaffney, 2001). The fibrinolytic system, a crucial defense mechanism against intravascular thrombosis, is controlled by the plasminogen activator system. The fibrinolytic process is mainly influenced by the dynamic interaction between tissue plasminogen activators (t-PA) which produce plasmin (from plasminogen) that promotes fibrinolysis, and its specific inhibitor, plasminogen activator inhibitor-1 (PAI-1) that inhibits the proteolytic activity (Harmening, 2009). Hypertensives have higher levels of plasma PAI-1 and t-PA antigen that have been shown to have predictive power for myocardial infarction, stroke and cardiovascular death (Juhan-Vague and Alessi, 1993;Ridker, 1994).

Several reports have shown that essential hypertension is associated with abnormalities in fibrinolytic balance caused by altered plasma levels of fibrinogen, PAI-1 and t-PA (Wall, 1995;Dell'Omo *et al.*, 2003). According to the study by Armas-Herna´ndez *et al* in 2007, findings of increasing PAI-1 and t-PA levels in relation to increasing BP are compatible with a decreased fibrinolytic state in hypertensive patients. Similarly, a comparative cross-sectional study by Wall *et al* reported that borderline hypertensive subjects had a higher concentration of t-PA levels than did control subjects, though no significant differences in t-PA activity or PAI-1 levels between groups (Wall, 1995). Moreover, the levels of PAI-1 and t-PA were positively related to SBP and DBP in a large Framingham Offspring cohort Study (n = 2652; 1193 men and 1459 women) of healthy middle aged men and woman (Poli, 2000).

Since both of t-PA and PAI-1 are synthesized in the vascular endothelium, endothelium dysfunction induces an imbalance in fibrinolysis (Coffey *et al.*, 2011). This can be demonstrated by the ratio of PAI-1/t-PA that tends to be higher in hypertensive cases. A study reported that the ratio of active t-PA to active PAI-1 is 1:50 in men with thrombotic disease as compared to the healthy male subjects, which is approximately 1:8 ratio (Lottermoser *et al.*, 1998). In another study, the ratio PAI-1/t-PA tends to be higher in hypertensive groups, but did not reach significance (Armas-Herna´ndez *et al.*, 2007).

1.2.5 D-dimer generation and clinical applications

A. D-dimer generation

D-dimer is a fibrin degradation product (FDP) generated by the sequential activity of three enzymes: thrombin, activated factor XIII (factor XIIIa), and plasmin (**Figure 2**). The process starts when thrombin, generated from prothrombin at the site of vessel injury upon activation of coagulation pathway, cleaves fibrinopeptide A with 16 amino acids and fibrinopeptide B with 14 amino acids from fibrinogen to form fibrin monomers (Adam *et al.*, 2009;Olson, 2015;Cimmino *et al.*, 2017). Fibrin monomers polymerize end to end and side to side spontaneously and then stable fibrin clot is formed when fibrin-stabilizing factor (factor XIII) covalently links the adjacent domains of three fibrin monomers, the D–D–E domains. The central part of the molecule is called the E domain while the two outer ends are the D domains (Olson, 2015;Riley *et al.*, 2016).

Owing to the parallel activation of the fibrinolytic system to maintain proper balance between coagulation and fibrinolysis, the cross-linked fibrin network undergoes plasmin-mediated degradation to form FDP (Adam *et al.*, 2009;Harmening, 2009). Fibrin-bound plasmin degrades the fibrin network into soluble fragments consisting of (DD)E which in turn is a complex containing D-dimer generated from cross-linked adjacent D domains (DD) noncovalently bound to fragment E (Riley et al., 2016). D-dimer antigen remains undetectable until it is released from crosslinked fibrin by plasmin mediated proteolysis of fragment E released from the (DD)E complex, and D-dimer containing two D domains (hence the name) formed. D-dimer then circulates in plasma with a half-life of approximately 8 hours until it is cleared by the kidneys and the reticuloendothelial system (Hager, 2009). D-dimer antigen as detected by commercially available assays can either be derived from the soluble fibrin

polymers before their uptake in the clot or be the product of plasmin cleavage of the fibrin clot (Adam *et al.*, 2009). Therefore, because it can only be generated when there is formation and degradation of cross-linked fibrin, D-dimer provides a global marker of activation of the coagulation and fibrinolytic systems, and serves as an indirect marker of thrombotic activity (Riley et al., 2016).

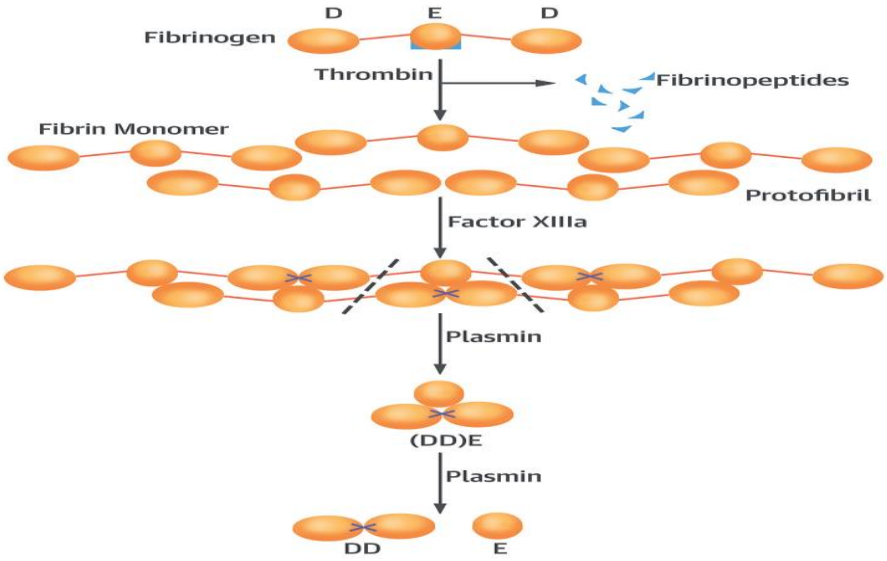

Figure 2:D-dimer generation from crosslinked fibrin *(Adam* et al., *2009).*

B. D-dimer clinical application

D dimer (D-DI) reflects the ongoing activation of the hemostatic system and it is a good biochemical marker of thrombogenesis and fibrin turnover (Lip, 1995). Several clinical scenarios might prompt a practitioner to measure or monitor D-DI levels. In general, D-DI test may be ordered to ascertain to what extent fibrin formation has been initiated or to learn whether there is any change in this process in the course of a specific therapy or disease process (Lowe, 2005). D-DI have intensively studied as potential specific diagnostic markers for thromboembolic disease among many other coagulation breakdown products (Riley *et al.*, 2016).

In practice, D-DI are exquisitely sensitive biomarkers for routine use in patients with Disseminated Intravascular Coagulation (DIC), Deep Venous Thrombosis (DVT) and Pulmonary embolism (PE)(Wells *et al.*, 2003). Apart from these, circulating D dimers are also elevated in patients with coronary artery disease, acute aortic dissection and other CVDs, cancer, trauma, pregnancy (rising two- to-fourfold by delivery), infectious and inflammatory

diseases, severe renal disease, recent surgical procedure, advanced age (limiting its use in those >80 years old), and many other conditions, health factors, and diseases (Chabloz *et al.*, 2001;Harper *et al.*, 2007;Adam *et al.*, 2009;Riley *et al.*, 2016). However, because the elevation is less specific than for DVT/PE, laboratory monitoring for D dimer has limited clinical usefulness in patients with these diseases (Adam *et al.*, 2009;Riley *et al.*, 2016). The role of D dimer in patients with other conditions such as predicting the risk of stroke in atrial fibrillation, identifying patients with coronary artery disease or human immunodeficiency virus (HIV) infection at risk for cardiovascular events, or for ruling out acute aortic dissection is uncertain (Hager, 2009).

1.2.6 D-dimer and Hypertension

Essential arterial hypertension often predisposes patients to a prothrombotic state and increased risk of vascular and organ complications (Tabak *et al.*, 2009). D-DI is a good biochemical marker of thrombosis and a high plasma levels of which is related to BP (Lip, 1995;Lip *et al.*, 1997;Tabak *et al.*, 2009). D-DI assay has high power of negative predictive value, meaning, finding a D-DI that is within the reference interval essentially excludes the diagnosis of VTE. Finding of high fibrin D-DI in patients with hypertension suggests ongoing intravascular fibrin formation (and possibly early thrombus formation) though elevated D-DI has little specificity and low positive predictive value(Olson, 2015). Plethora of studies have shown that high plasma fibrin D-DI levels (even within the normal range) are predictive of both arterial thrombotic events (Fowkes, 1993;Ridker *et al.*, 1994;Lowe, 1995) and postoperative thrombosis (Rowbotham, 1992); this suggests that increased fibrin turnover may perhaps be a continuum between health, statistically increased fibrin turnover as a prethrombotic state, and overtly increased fibrin turnover in acute thrombosis.

Several number of studies have found that elevated plasma D-DI levels in essential hypertensive patient (Vaziri, 1993;Lip *et al.*, 1997;Sechi *et al.*, 2000;Coban *et al.*, 2004) and white coat hypertension (WCHT) groups (Coban *et al.*, 2004). According to the Sechi *et al* (2000), D-DI concentrations is significantly related to the severity of TOD in hypertensive patients. This got support from the study done by Zhang and his colleagues who demonstrated that D-dimer is related to different levels of BP, dangerous states and TOD; Antihypertensive drugs; enalapril and terazosin could reduce the level of BP and hence decrease the D-dimer

level (P <0.01) (Zhang, 2003). Similarly, in one prior study in hypertensive patients with left ventricular hypertrophy, left ventricular enlargement, and left atrial enlargement, higher levels of D-dimer were detected (Chi, 2002). A community-based follow up study showed that D-dimer levels increased with age and functional disability and among the health variables, only high BP was predictive of D-dimer level, supporting the correlation D-dimer and BP (Pieper *et al.*, 2000). A study done in Sudan reported that D-dimer levels are higher among hypertensive patients than in controls and indicated the hypercoagulable state among hypertensive patients (Ibrahim and Abdalla, 2014). As a precedence to this study, in Sudan, it was shown that the D-dimer level increased in hypertensive patients when compared with a healthy control groups though it was insignificant (Osman and Muddathir, 2013).

1.3 STATEMENT OF THE PROBLEM

Hypertension is one of the leading causes of the global burden of disease. Though the burden of the disease varies considerably between regions of the world, hypertension is considered to be the main contributor to increased cardiovascular morbidity and mortality(Chobanian *et al.*, 2003). Approximately 7.6 million deaths (13–15% of the total) annually and 92 million disability adjusted life years (DALYs) worldwide were attributable to high BP in 2001(Chobanian *et al.*, 2003). The proportion of the global burden of disease attributable to hypertension has significantly increased from about 4.5 percent (nearly1 billion adults) in 2000 (Kearney, 2005), to 7 percent in 2010(Lim, 2012). In US, the absolute burden of hypertension is consistently increased, from 87.0 million in 1999–2000 to 108.2 million in 2015– 2016 in the US adult population aged ≥20 years (Carey and Whelton, 2018).

The prevalence of hypertension has also been increasing in developing countries (Bygbjerg, 2012). In Ethiopia, the prevalence of hypertension is higher and has been increasing according to different small-scale studies and a national level meta-analysis that summarized a number of small-scale studies done in different part of Ethiopia (Awoke *et al.*, 2012;Kibret and Mesfin, 2015;Asresahegn *et al.*, 2017).

Hypertension, an independent risk factor for developing TOD, doubles the risk of stroke, coronary heart disease (CHD), Peripheral Arterial Disease (PAD) and End Stage Renal Disease (ESRD) (Chobanian *et al.*, 2003;Foe¨x and Sear, 2004). Moreover, hypertension is a silent killer mostly as a consequence of the associated complications. This is due to the

inability to early detect TOD as most hypertensive patients are asymptomatic and detected incidentally when they come to hospital for other health seek. Even after the hypertension is diagnosed, there is no well-studied biochemical risk marker routinely used to predict or early detect the associated thromboembolic disease and hence to determine the severity of hypertension (Asresahegn *et al.*, 2017). This makes hypertension the single most important cause of morbidity and mortality globally, and highlights the urgent need of action to address the problem (Beaglehole *et al.*, 2008).

Hypertension, independently or by clustering with the coexisting risk factors such as age, gender, smoking, obesity, diabetes, and dyslipidemia, is involved in the pathogenesis to develop TOD (MacMahon, 1990); with complications predominantly occurring as a result of thrombotic rather than hemorrhagic*(*Beevers *et al., 2001).* Evidence is accumulating that indicates that the hemostatic imbalance predicts future vascular events both in ischemic heart disease and stroke resulting from thrombi or emboli induced vascular occlusion. Thus, individuals who present with impaired hemostatic/fibrinolytic activity tend to have increased risk for ischemic cardiovascular events (Gleerup, 1991).

The potential role of many haemostatic factors including fibrinogen, factor VII, D-dimer, prothrombin fragment 1 + 2, factor VII, vWF, t-PA and PAI-1 associated with atherogenesis and/or thrombosis and increased risk for CVD have been investigated (Ruberg, 2002).

D-DI is reportedly a good independent biochemical risk marker of thrombogenesis and fibrin turnover, and is often used as a marker for DIC, DVT and PE (Wells *et al.*, 2003). In patients without evidence of coagulopathy, the D-DI may represent microvascular thrombosis and the elevated levels of which may provide clinical utility in predicting risk of future myocardial infarction, stroke, and PAD in the general population (Fowkes, 1993;Smith, 1997).

Thus, risk of CVD resulting from hypercoagulability due to endothelial injury in hypertensive disorder can be proven by measuring D-DI level which is more sensitive, highly predictive and noninvasive procedure. Therefore, the aim of this study was to assess plasma D-DI level among hypertensive patients and to see its correlation to the severity of disease in a group of patients with essential arterial hypertension and compare the values obtained with a group of healthy normotensive subjects.

1.4 SIGNIFICANCE OF THE STUDY

The global burden of disease attributable to hypertension is significantly increasing and the morbidity and mortality of which is associated with increased risk for CVD (Ruberg, 2002). Prior studies have evaluated the association of hemostatic factors (including D-dimer) and hypertension, and some of which have reported an elevation in the D-DI levels among hypertensive patients (Lip *et al.*, 1997;Zhang, 2003;Ibrahim and Abdalla, 2014). However, these studies require confirmation through further studies to use D-DI test routinely as risk marker in hypertensive patients and also there was no study that has elucidated specifically the relationship between severity of hypertension and plasma D-DI level.

Therefore, this study was done to ascertain whether plasma D-DI levels can be useful indicator for hypertension severity; It specifically investigates whether patients with elevated plasma D-DI level have more severe hypertension compared to normal level D-DI hypertensives.

Besides, it is possible in the future that D-DI test may help predict individual risk for CVD among hypertensive patients. This early prediction and detection using D-DI test in turn may improve the prognosis and quality of life as well as may lower DALYs and prevent premature death caused by cardiovascular complications.

In addition, the findings from this study provide new insight to see the problem for better intervention and help to shape clinical as well as public health care of the patients and the general population. Moreover, the results obtained from this study may also be used as baseline or reference to pave the way for conducting further related studies.

Although the relationship between hypertension and D-DI level has been reported previously and gained great interest among researchers worldwide in recent years, to the best of our knowledge, studies addressing the severity of hypertension and D-DI level are rare worldwide and is the first of its kind in Ethiopia.

1.5 HYPOTHESIS

The null hypothesis of this study was (1) plasma D-dimer levels in patients with hypertension is not higher than in normotensive individuals and (2) there is no observable correlation between plasma D-dimer level and disease severity in hypertensive patients.

2. OBJECTIVES

2.1. General objective

- Assessment of plasma D-dimer level and its correlation with disease severity among hypertensive patients at Y12HMC, Addis Ababa, Ethiopia, 2019.

2.2. Specific objectives

- To determine the plasma D-dimer levels of hypertensive patients
- To compare D-dimer level in hypertensive patients with apparently healthy individuals
- To compare D-dimer level between groups of different independent variables
- To evaluate the correlation between plasma D-dimer level and the severity of hypertension
- To identify factors affecting plasma D-dimer levels among hypertensive patients
- To assess factors associated with hypertension
- To determine predictive ability of plasma D-dimer level to differentiate between complicated and non-complicated hypertension

3. METHODS AND MATERIALS

3.1 Study area and period

This study was conducted in Yikatit 12 Hospital Medical College (Y12HMC) in Addis Ababa from May 03 to June 09, 2019. Yikatit 12 Hospital (Y12H) is a specialized hospital located in Arada Sub City of Addis Ababa. It was established in 1923 as Haile Selassie I Hospital which later, after 20 years, in 1975 was named into Y12H. Since 2011, the hospital was renamed as Y12HMC and now it is one of teaching hospital for both clinical and preclinical training. A total of 1,134 health professionals are working recently according to the information from the Y12HMC administrative office and the hospital gives service in more than 36 areas. The hospital is serving for more than 5 million people in the catchment area in all six major departments and other units. This hospital gives service for about 200 to 250 patients per day in all Outpatient Department (OPD) units. Therefore, this study was specifically conducted at medical OPD hypertension clinic of Y12HMC.

3.2 Study design

A hospital based comparative cross-sectional study design was employed.

3.3 Population

3.3.1 Source population

All hypertensive patients visiting Y12HMC and all healthy individuals aged 20-80 years old.

3.3.2 Study population

All eligible hypertensive patients attending Y12HMC during the study period as well as all controls who were eligible and volunteer to participate in the study.

3.4 Eligibility criteria

3.4.1 Inclusion criteria

All volunteer hypertensive patients attending hypertension clinic of the Y12HMC during the study period were included in this study while all volunteer healthy individuals who didn't have diseases or conditions mentioned in exclusion criteria were taken as control.

3.4.2 Exclusion criteria

Study participants who had the following diseases or conditions were excluded from the study:

These diseases or conditions were excluded by reviewing the patient's card and historically from the participants.

- Suspected thromboembolism (DVT and PE)
- Disseminated intravascular coagulation (DIC)
- Recent surgery/trauma (in the last 3 months)
- Pregnancy (from the medical records or reported from the participant)
- Early age (<20 year) and advanced age (>80 year)
- Known history of diabetes mellitus
- Known history of renal failure
- Liver disease
- Anticoagulatory (heparin or warfarin) or thrombolytic treatment (streptokinase or urokinase).

3.5 Sample size determination and sampling techniques

3.5.1 Sample size determination

Sample size was calculated using single population proportion formula based on the assumption of 19.6% prevalence of hypertension in Ethiopia (Kibret and Mesfin, 2015).

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

Where n is minimum sample size required; $Z_{1-\alpha/2}$ is the standard normal variable at (1-α) % confidence level and at α level of significance. Usually 95% confidence level is used = 1.96; P is estimate of the prevalence rate of hypertension in the population is the margin of sampling error tolerated, assumed 0.05. Therefore, the calculated sample size (n) was 242.

However, due to budget constraint this wasn't feasible. Besides, many studies done internationally on D-dimer used a smaller sample size than the calculated one; mostly they used less than 100 participants. Therefore, 60 hypertensive patients and 40 normal individuals were included in the study in accordance with the meager research support obtained.

3.5.2 Sampling techniques

Purposive non probability sampling technique was used to include all eligible hypertensive patients who visited the hospital during the study period.

3.6 Study variables

3.6.1 Dependent (criterion)variable

- Plasma D-dimer level

3.6.2 Independent (predictor) variables

- Socio demographic factors (age, sex, marital status, residence, occupation, education)
- Behavioral factors (smoking, alcohol use, physical activity)
- Family history of hypertension
- Anthropometric parameter (BMI, weight, height)
- Clinical factors (BP, duration of HTN, no. of drug therapy, presence of complication)

3.7 Operational Definition

📖 **Blood pressure (BP) classification:** Based on the Ethiopia's guideline and other literatures, BP was classified into two groups: Normotension (BP<140/90) and hypertension (≥140/90) (Chobanian *et al.*, 2003;Wubaye *et al.*, 2016;Muela *et al.*, 2017).

📖 **Hypertension (HTN) severity:** Since BP measurements alone while on anti-hypertensive regimen have been considered a less reliable indicator of hypertension severity, the combination of BP levels**,** number of drugs taken (not fixed drug combinations) and presence of complication were used to indicate the disease severity of adult hypertensives (aged ≥20 years) and were divided into three clinical stages; stage 1 HTN if BP was 140/90-159/99 or taking one or two antihypertensive medications; stage 2 HTN if BP≥160/100 or taking three or more medications and severe HTN if BP≥180/120 or complication present (Chobanian *et al.*, 2003;Wubaye *et al.*, 2016;Muela *et al.*, 2017). The study comparatively analyzed D-DI level between normotension and HTN as well as between stage 1, stage 2 and severe HTN.

📖 **Antihypertensive drugs:** are class of drugs that are used to lower high BP in hypertensive patients. Diuretics, beta blockers, vasodilators, angiotensin converting enzyme inhibitors (ACEi), angiotensin II receptor blockers (ARBs) and calcium channel blockers (CCBs) are the most widely used medications**.** Based on the number of antihypertensive drugs the

patient was taking, medication can be monotherapy; if a patient was taking one medication and dual therapy, triple therapy, or quadruple therapy if patient was taking two, three, or four antihypertensive drugs respectively (James *et al,* 2014).

- **Well controlled blood pressure (BP):** keeping the systolic blood pressure (SBP) of hypertensive patients below 140 mmHg and diastolic blood pressure (DBP) less than 90 mmHg, by using pharmacological (using antihypertensive drug(s)) and/or non-pharmacological treatment (life style modification) (Alemu *et al.,* 2017).
- **Uncontrolled or poorly controlled blood pressure (BP)**: blood pressure not well controlled despite the life style modification and/or prescribed antihypertensive drugs i.e. SBP 140 mmHg or above and/or DBP 90 mmHg or above (Alemu *et al.,* 2017).
- **Smoking status** was defined as follows(Asresahegn *et al.,* 2017)
  - **Current (active) smoker**: Those who have smoked greater than 100 cigarettes in their life time and have smoked in the last 28 days.
  - **Prior (previous) smoker:** Those who have smoked greater than 100 cigarettes in their life time but haven't smoked in the last 28 days.
  - **Non-smoker**: Those who haven't smoked greater than 100 cigarettes in their life time and don't currently smoke.
- **Alcohol consumption:** Women who consume two or more alcoholic beverages(beers) per day and men who have three or more drinks (beers) per day were considered as alcohol consumers (Wubaye *et al.,* 2016).
- **Regular physical exercise:** Individuals who take at least 30 minutes of moderate physical activity (e.g. brisk walking, cycling) a day for at least 5 days a week (Wubaye *et al.,* 2016).
- **Complicated hypertension:** the patient has thromboembolic complication (like stroke, heart disease (myocardial infarction, left ventricular hypertrophy, cardiac arrythmia or CAD), secondary renal failure, peripheral arterial disease) developed as a result of hypertension as per the documentation of the patient's card or from patient's history.
- **Deep Venous Thrombosis (DVT):** Thrombus formation at lower extremities diagnosed by physicians either clinically or through laboratory testing or ultrasound imaging documented in patient's card (Wells *et al.,* 2003).

- **Pulmonary Embolism (PE):** is a potentially fatal cardiovascular disease usually caused by DVT and diagnosed clinically besides the laboratory and imaging findings documented in patient's card (Kabrhel *et al.*, 2010)**.**
- **Disseminated intravascular coagulation (DIC):** is a syndrome of increased propensity for clot formation triggered by a pathological stimulus that disrupts the coagulation balance resulting in a fibrin clot that disseminates or spreads throughout the microcirculation, diagnosed by laboratory testing documented in the medical record (Furie, 2005).

3.8 Data and Specimen collection procedure

After informed consent was obtained from patients, all necessary information regarding sociodemographic, medical and other data were collected from the selected participants using structured questionnaire by two nurses working at Y12HMC hypertension clinic under the supervision of the investigator and another trained supervisor through face to face interview, reviewing patient's card and direct measurement of the variables like weight, height, and BP.

3.8.1 Blood pressure measurement

BP was measured in the morning (before taking antihypertensive drug) at sitting positions from right arm in a quiet room using an Omron automatic device after making patient comfortable and after 15 minutes of rest. A mean of 3 measurements was taken and used to determine SBP and DBP in each study participant.

3.8.2 Anthropometrical measurement

The weight of the hypertensive patients was measured using a standard balance while the height was measured using a height measuring scale with light clothing and without shoes. Body Mass Index (BMI) was then calculated based on the formula: BMI=Weight (in kg)/(Height in m^2) (Tambe *et al.*, 2010). Using the De Lorenzo *et al* classification(De Lorenzo *et al.*, 2016), four categories of BMI were identified: underweight, <18.5 kg/m^2; normal, 18.5–24.9kg/m^2; overweight, 25.0–29.9kg/m^2; and obese, ≥30 kg/m^2.

3.8.3 D-dimer Specimen preparation and Biochemical assays

Blood sample collection and laboratory analysis were done with the assistance of two experienced laboratory technologists working at Y12HMC and Ethiopian Public health institution (EPHI) respectively. Measurement of D-dimer level was done using fully

automated high-sensitive quantitative D-dimer chemistry analyzer at National Reference Laboratory for Clinical Chemistry of EPHI.

Principles: To detect plasma D-dimer antigen, particle enhanced immunoturbidometric assay has been developed, which utilize antibodies reacting with epitopes specifically present on the D-dimer molecule. These epitopes are generated as a result of factor XIII's cross linkage of fibrin polymers, and are not found on other FDPs. First, latex particles of uniform size are coated with monoclonal antibodies (F(ab')2 fragments) to the D-dimer epitope. The antigen/antibody complexes produced by the addition of samples containing D-dimer lead to an increase in the turbidity of the test reactants. The change of absorbance with time is dependent on the concentration of D-dimer epitopes in the sample. The precipitate is determined turbidimetrically.

Materials and equipment: 5cc syringe, EDTA tube, centrifuge, micropipette, Nunc tube, refrigerator, microtiter plate, sample tube, reagents (R1: TRIS/HCl buffer 250 mmol/L, pH 8.2; preservatives; R2; latex particles coated with monoclonal anti-human D-dimer antibodies (mouse) 0.12 %; preservative) and chemistry analyzer.

Procedures: After informed consent was obtained, 4 ml of venous blood was drawn from each overnight fasted participant by venipuncture from the antecubital vein and was poured into EDTA tubes by an experienced Laboratory Technologists. The blood was thoroughly mixed with the anticoagulant inside the tube. Blood was then taken to the Biochemistry Laboratory Unit and centrifuge for 15 minutes at 3,000 rpm within 4 hours after collection at room temperature to obtain supernatant or platelet-poor plasma (PPP). Plasma was separated from cells into plane container called Nunc tube using micropipette and then PPP was stored and refrigerated at -20^{o}C until assayed. One ml aliquot plasma was taken into sample tube for analysis and plasma fibrin D-dimer was measured by fully automated immunoturbidometric assay, which is an accurate and sensitive quantitative assay method, using a Roche COBAS INTEGRA 6000 analyzer using the Tina-quant D-dimer Gen.2 test. The Tina-quant D-dimer test was then performed using a cutoff of 0.5 mg FEU/l.

3.9 Data processing and Statistical analysis

After data was collected and coded, it was checked for completeness, cleaned and entered to computer and statistically analyzed using Statistical Package for the Social Sciences (SPSS)

version 25.0. The data was presented using table, graphs and charts. Proportions and summary statistics such as mean and standard deviation were calculated for quantitative variables, unless otherwise stated. Chi square and Fisher's exact test (if value<5 in one cell) was done for categorical variables, when appropriate. Independent samples t-test and Wilcoxon Mann Whitney U-tests were used to see the difference in the mean values of continuous variable between hypertensive and control groups as well as between study variables with two responses, depending on whether a variable fit the assumptions. One-way ANOVA and Kruskal-Wallis tests were also used to see the difference in the mean values of D-dimer level among the three stages of hypertension and other study variables with three or more responses. The correlation between continuous variables that met the assumptions were computed using the Pearson's correlation coefficient. A point-biserial correlation coefficient was computed for the dichotomous variables. Correlations between the ordinal variables and continuous variables that don't meet the assumptions were assessed by Spearman nonparametric test. Simple and multivariate regression analyses were performed to examine the predictive variables and odds ratio. A binary logistic regression model was used to examine factors associated with hypertension while linear regression model was used to analyze the predictor variables associated with plasma D-dimer level. The variables that were found with P<0.2 at bivariate regression analysis were eligible variables to fit the final regression model and were entered to multivariate regression model. Adjusted Odds Ratio (AOR) with 95% confidence interval (CI) was used to show the strength of association. Those variables with a two-sided P-values of less than 0.05 were considered as statistically significant. The diagnostic or predictive performance of D-dimer levels for hypertension related complications was evaluated using a receiver operating characteristic (ROC) curves analysis. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the D-dimer test were calculated using a cut-off value that was selected from the ROC curve.

3.10 Data quality control and management

The data quality management started during questionnaire development by translating the questionnaires prepared in English (by reviewing different literatures) into the local Amharic language. Before data collection, training was given for data collectors and supervisor regarding the objective of the study, inclusion and exclusion criteria, which patient group involved in the study and others. Assigning these experienced (trained) data collectors and

supervisor improved the data quality during data collection. Data quality was also assured during blood sample collection by strictly following the standard aseptic operational procedure. The kit was made free from contamination and check for consistency. Laboratory analysis was done following the appropriate procedures based on the manufacturer's instruction. All the laboratory procedures were handled with the assistance of professional laboratory technologists and results were checked for completeness by the supervisors to maintain the overall quality of data. During data entry and analysis using computer software, due attention was given to keep the data quality.

3.11 Ethical consideration

Ethical clearance was obtained from Research Ethical Committees of Biochemistry Department, College of Health Sciences, Addis Ababa University after review was conducted, and approval was obtained by a letter DRERC 04/14 and meeting number DRERC 01/119. A formal collaboration letter for data collection was obtained from the Department of Biochemistry to Y12HMC. The purpose and objective of the study were explained briefly and written informed consent was obtained from each eligible study participants before the data and blood sample were collected. Participants were also informed verbally that participation is on voluntary basis and they were assured on the right to refuse blood sample and data collection. Confidentiality of the information taken by laboratory technologists from study participants was kept secret and code numbers were used during sample collection.

4. RESULT

4.1 Socio demographic characteristics

A total of 100 study participants (60 hypertensive cases and 40 healthy controls) were included in this study. The mean (±SD) age of hypertensive patients and healthy controls was 57.2 ±12.2 and 36.3±12.4 years with a minimum age of 30 and 20 years and a maximum age of 80 and 63 years respectively. Most of the participants were females in both hypertensives 37/60(61.7%) and healthy controls 22/40 (55.0 %). All hypertensives were urban dwellers while most 38/40(95.0%) of the healthy controls were living in urban. More than three quarters 46(76.7%) of hypertensives and about three in five 24(60.0%) controls were married. With regard to educational status, 18(30.0%) hypertensives attended primary school whereas 22(55.0%) of healthy controls completed college or university. Concerning occupation, 24(40.0%) of cases and 24(60.0%) of controls were government workers (**Table 2**).

Table 2:Socio-demographic profile of hypertensive and control groups

Variable	Category	N (%)	
		Hypertensive (60)	Control (40)
Age	20-29	0 (0.0)	18(45.0)
	30-39	5(8.3)	7(17.5)
	40-49	9(15.0)	7(17.5)
	50-59	18(30.0)	7(17.5)
	60-69	15(25.0)	1(2.5)
	70-80	13(21.7)	0(0.0)
Sex	Male	23(38.3)	18(45.0)
	Female	37(61.7)	22(55.0)
Marital status	Single	2(3.3)	15(37.5)
	Married	46(76.7)	24(60.0)
	Divorced	6(10.0)	1(2.5)
	Widowed	6(10.0)	0(0.0)
Educational status	Illiterate	14(23.3)	1(2.5)
	Primary school	18(30.0)	5(12.5)
	Secondary School	14(23.3)	12(30.0)
	College/University	14(23.3)	22(55.0)
Occupational status	Housewife	19(31.7)	5(12.5)
	Merchant	6(10.0)	1(2.5)
	Car driver	2(3.3)	2(5.0)
	Gov.t employee	24(40.0)	24(60.0)
	Private employee	5(8.3)	4(10.0)
	Daily laborer	3(5.0)	4(10.0)
	Other	1(1.70	0(0.0)

N (%) indicates the frequency and percentage

4.2 Risk factors of Hypertension

Smoking, alcohol consumption, physical activity, family history of hypertension (HTN) and anthropometric parameters (weight, height and BMI) of the study participants are traditional risk factors that were investigated in this study. The majority of participants (93.3% cases and 97.5% controls) never smoked cigarette. Most hypertensives 58(96.7%) and healthy individuals 38(95.0%) didn't drink alcohol excessively. Only 16 (26.7%) of the recruited hypertensive cases and 12(30.0%) of healthy controls were regularly doing physical exercise. Eighteen (30.0%) hypertensives and ten (25.0%) healthy individuals were having family history of HTN (**Table 3**).

Table 3: Risk factors of hypertension among study participants

Variable	**Category**	**N (%)**	
		Hypertensive (60)	Control (40)
Smoking status	Never	56(93.3)	39 (97.5)
	Prior smoker	2(3.3)	0(0.0)
	Active smoker	2(3.3)	1(2.5)
Alcohol Consumption	Yes	2(3.3)	2(5.0)
	No	58(96.7)	38(95.0)
Physical activity	Yes	16(26.7)	12 (30.0)
	No	44(73.3)	28(70.0)
Family history of hypertension	Yes	18(30.0)	10 (25.0)
	No	42(70.0)	30(75.0)

N (%) indicate the frequency and percentage

With regard to anthropometric parameters, as shown in **Table 4**, the mean ± SD of weight, height and BMI of hypertensives were 68.7±14.13, 1.6±0.09 and 26.5±4.96 respectively whereas that of healthy controls were 62.3±11.12, 1.6±0.11 and 23.5±4.67 respectively. Independent sample t-test showed that weight and BMI of hypertensive groups were found to be significantly higher compared to that of healthy control groups. But the Mann-Whitney U-test showed that there was no significant height difference between the two groups.

Table 4:Anthropometric parameters in hypertensive cases and control groups

Anthropometry	**Mean ±SD**		**P-value**
	Hypertensives (60)	Controls (40)	
Weight	68.7±14.13	62.3±11.12	**0.017**
Height	1.6±0.09	1.6±0.11	0.234
BMI	26.5±4.96	23.5±4.67	**0.003**

P-value in bold are significant (P<0.05)

Based on Body Mass Index (BMI) classification, majority 34 (56.7%) of hypertensives were overweight 16 (26.7%) and obese 18 (30.0%) while only 13(32.5%) of controls were overweight 8 (20.0%) and obese 5 (12.5%) (**Figure 3**).

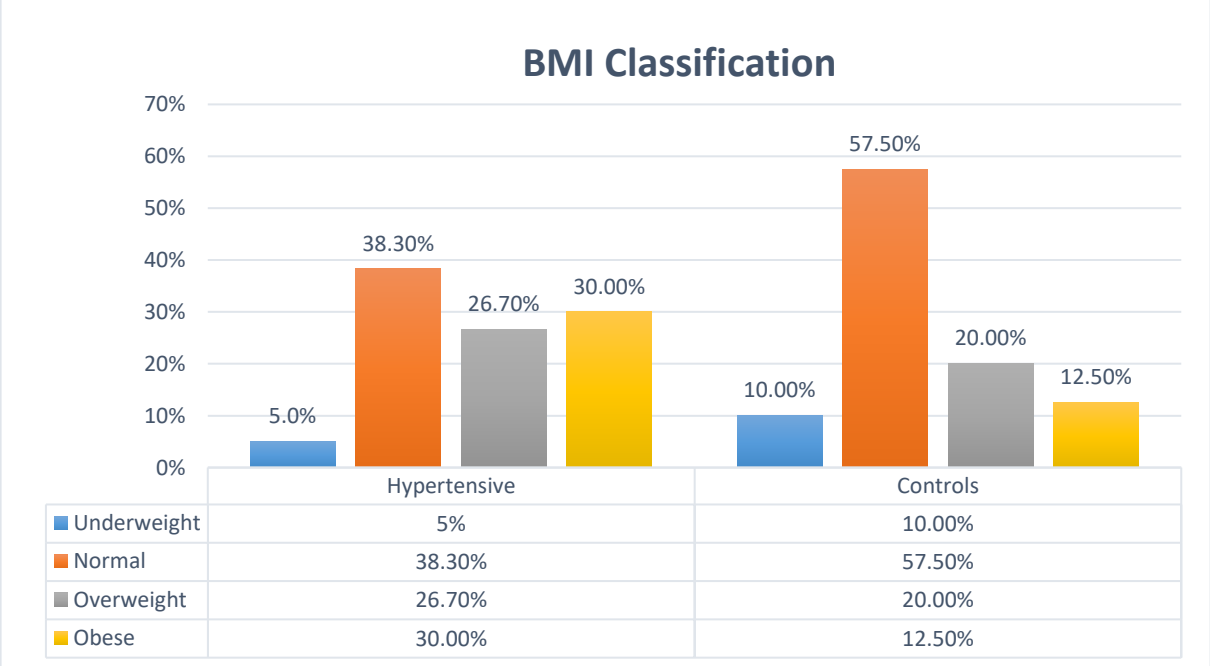

Figure 3:BMI classification among hypertensive patients and healthy controls

4.3 Clinical characteristics of hypertensive patients and healthy controls

According to this study, stage1 HTN accounted for the largest proportion 30(50.0%) among hypertensive patients while stage 2 and severe HTN accounted for the remaining 14(23.3%) and 16(26.7%) respectively. The mean (±SD) SBP and DBP of hypertensive patients were 140.7±18.1and 84.5±10.5 respectively. Sixteen (26.7%) of the total 60 hypertensive cases were complicated with stroke 11(18.3%) or heart diseases 5(8.3%). All hypertensive patients were taking at least one type of antihypertensive drug in which monotherapy took 24(40.0%) and the rest significant proportion of patients were on dual therapy, triple therapy and quadruple therapy, which accounted for 41.7%, 15.0%, 3.3% respectively. Concerning healthy controls, the mean SBP was 111.9±11.97, which ranges from 80 to 130 while the mean DBP was 75.1±6.84, ranges from 60 to 88. Among hypertensives, the duration of HTN ranges from 1 month to 28 years with mean duration of 5.7±5.3years. More than half 33(55.0%) of hypertensive patients have poorly controlled BP (**Table 5**).

Table 5:Clinical characteristics of Hypertensive patients

Variables	**Hypertensives (60)**		
Severity of hypertension, n (%)	Stage 1 Hypertension		30(50.0)
	Stage 2 Hypertension		14(23.3)
	Severe Hypertension		16(26.7)
SBP, mean ± SD, mmHg	140.7±18.1		
DBP, mean ± SD, mmHg	84.5±10.5		
Duration of HTN, mean ± SD, year	5.7±5.3		
No. of antihypertensives, n (%)	Monotherapy		24(40.0)
	Dual therapy		25(41.7)
	Triple therapy		9(15.0)
	Quadruple therapy		2(3.3)
Complication, n (%)	Yes	Stroke	11(18.3)
		Heart disease*	5(8.3)
		Renal failure	0(0.0)
		PAD	0(0.0)
	No		44(73.4)
BP control, n (%)	Well Controlled		27(45.0)
	Poorly controlled		33(55.0)

*Categorical variables presented as frequency and percentages, n (%) and continuous variables expressed as mean ± SD. *****Hypertensive heart disease (HHD), Ischemic heart disease, LV hypertrophy.*

4.4 Results of Biochemical Analysis

4.4.1 Plasma D-dimer levels were significantly elevated in hypertensive patients

The plasma concentration of D-dimer (D-DI) in hypertensive patients and healthy control groups were examined. The concentrations of D-DI exceeded the normal range (>0.5mg/l FEU) in 38(63.3%) of hypertensives and 8(20.0%) of controls. From independent sample t-test, we observed a significantly higher ($p<0.001$) mean value of D-DI levels among hypertensives (1.1±2.0 mg/l) compared to control groups (0.37±0.3mg/l) (**Table 6**).

Table 6:Plasma D-Dimer levels between the hypertensive patients and controls

	Control (40)	Case (60)	*P-value*
D-DI (mg FEU/l), mean ±SD	0.37±0.3	1.1±2.0	*<0.001**

**Significant in t-test*

4.4.2 Comparison of D-DI levels between different independent variables using t-test

Independent sample t-test, shown in **Table 7**, showed that there were statistically significant differences in plasma D-DI levels between well controlled and poorly controlled hypertensive patients, in which, compared to the well-controlled hypertensive patients, poorly controlled hypertensives had significantly higher mean levels of plasma D-DI ($p<0.05$). In addition, our result showed that complicated hypertensive cases had significantly elevated plasma D-DI level than uncomplicated cases ($p<0.001$).

Table 7: Comparison of D-DI levels in hypertensives between groups of independent variables.

Variables		**D-DI level, mean ± SD, mg/l**	**P-value**
Sex	Male	0.96±0.75	0.346
	Female	0..86±0.71	
Alcohol consumption	Yes	1.46±0.71	0.149
	No	0.88±0.72	
Physical activity	Yes	0.84±0.81	0.432
	No	0.92±0.70	
Family history of HTN	Yes	0.96±0.71	0.606
	No	0.87±0.73	
BP control status	Well controlled	0.73±0.83	**0.029**
	Poorly controlled	1.14±0.63	
Presence of Complication	Yes	1.60±0.87	**<0.001**
	No	0.65±0.46	

P-values written in bold are significant (2-tailed)

4.4.3 Comparison of D-DI levels between different independent variables using ANOVA

The one-way analysis of variance (ANOVA) showed statistically significant differences among different age groups in the mean values of plasma D-DI level ($p < 0.05$). Apart from this, BMI, BP and number of antihypertensive drugs were significantly different among between groups in their mean plasma D-DI levels ($p<0.05$). The average levels of the plasma D-DI across different independent variables are depicted in **Table 8** and **Figure 4**.

Table 8:ANOVA of D-DI levels (mg/l FEU) according to different independent variables

		Sum of Squares	Df	Mean Square	F	P-value
Age group						
Plasma D-dimer	Between Groups	6.292	5	1.258	3.578	**0.035[a]**
	Within Groups	33.058	94	.352		
Marital status						
Plasma D-dimer	Between Groups	2.560	3	.853	2.227	0.090
	Within Groups	36.790	96	.383		
Educational status						
Plasma D-dimer	Between Groups	1.586	3	.529	1.344	0.265
	Within Groups	37.764	96	.393		
Occupation						
Plasma D-dimer	Between Groups	2.636	7	.377	.944	0.477
	Within Groups	36.714	92	.399		
Body Mass Index (BMI) classification						
Plasma D-dimer	Between Groups	5.133	3	1.711	4.800	**0.004[b]**
	Within Groups	34.217	96	.356		
Smoking						
Plasma D-dimer	Between Groups	.220	2	.110	.273	0.762
	Within Groups	39.130	97	.403		
Blood Pressure (BP) classification						
Plasma D-dimer	Between Groups	5.403	2	2.702	7.720	**0.001[c]**
	Within Groups	33.947	97	.350		
Number of drugs						
Plasma D-dimer	Between Groups	4.041	3	1.347	2.842	**0.025[d]**
	Within Groups	26.541	56	.474		

*From Tukey HSD Post hoc analysis: **a**-indicates significant difference ($p<0.05$) in D-DI level between 20-29 and 70-80 years age; **b**-significant difference in D-DI level between obese vs normal; **c**-indicate significant difference in D-DI level among all groups except normotensive vs stage 1 HTN; **d**-significant difference between mono- vs quadruple therapy.*

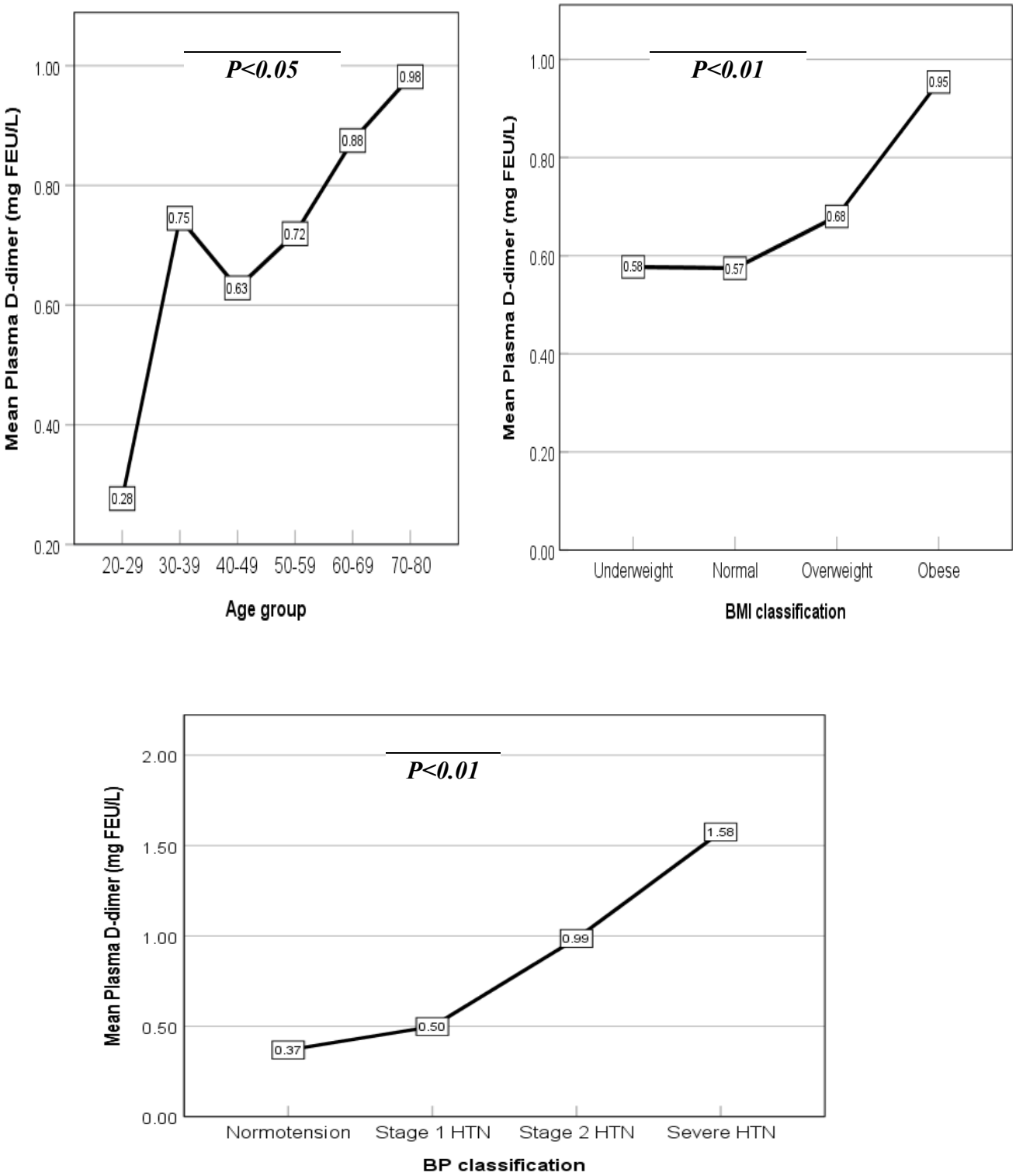

Figure 4: Mean plot showing the relationship between mean plasma D-DI levels with age group, BMI and BP classification.

4.4.4 Comparison of D-DI levels according to the severity of Hypertension

This study showed that plasma D-DI levels were increased significantly with the severity of HTN ($p<0.001$), in which the mean D-DI level of severe HTN (1.6 ± 0.87mg FEU/l) were significantly higher compared to both stage 1 HTN (0.50±0.25 mg FEU/l) and stage 2 HTN (0.99±0.63 mg FEU/l) from one way ANOVA as shown in **Table 9** and **Figure 5**.

Table 9:Comparison of Plasma D-Dimer according to the severity of hypertension

	Stage 1 HTN (30)	*Stage 2 HTN (14)*	*Severe HTN (16)*	*P-value*
D-DI (mg FEU/l), mean ±SD	0.50±0.25	0.99±0.63	*1.6±0.87*	*<0.001***

*** Significant in F-test*

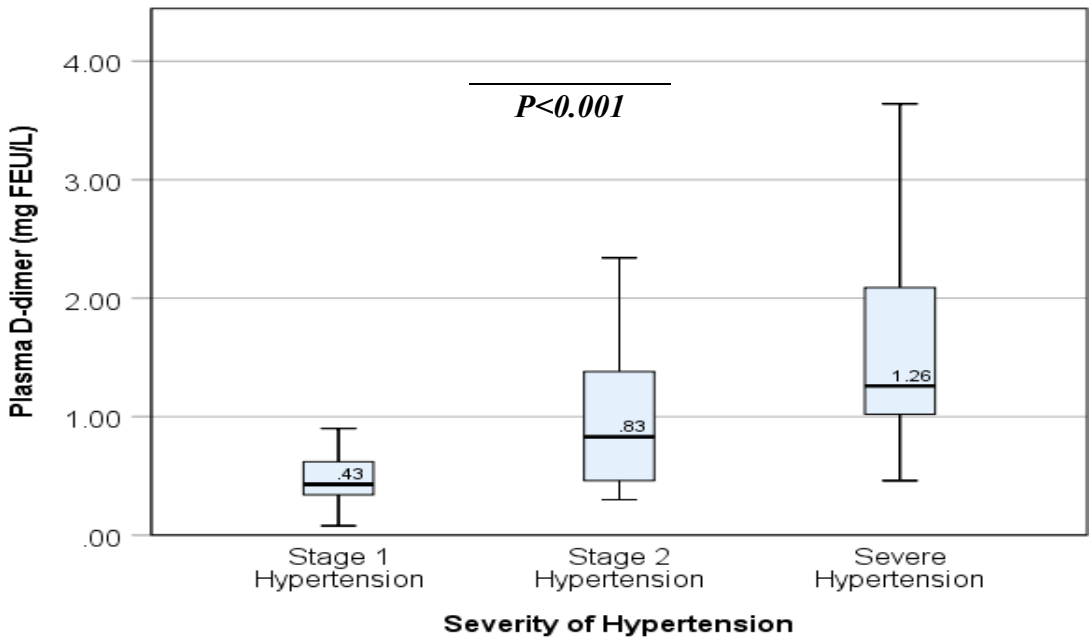

Figure 5: Box and Whisker plot showing the trend of D-DI Levels across the severity of HTN

For comparing two groups using one-way ANOVA and to screen out between which pairs differences exist, the Tukey HSD post hoc analysis were conducted and showed that there was statistically significant difference ($p<0.05$) in mean plasma D-DI level in any of pair combinations among the clinical stages of HTN i.e. between stage 1 HTN versus stage 2 HTN and severe HTN as well as between stage 2 HTN and severe HTN (**Table 10**).

Table 10:Tukey HSD Post hoc analysis for pairwise multiple comparison of plasma D-DI across clinical stages of HTN

Severity of HTN (I)	Severity of HTN (J)	Mean Difference (I-J)	Std. Error	Sig.	95% CI
Stage 1 Hypertension	Stage 2 HTN	-.48705*	.1842	**0.028**	(-.9304, -.0437)
	Severe HTN	-1.07633*	.1762	***<0.001***	(-1.5004, -.6523)
Stage 2 Hypertension	Stage 1 HTN	.48705*	.1842	**0.028**	(.0437, .9304)
	Severe HTN	-.58929*	.2083	**0.017**	(-1.0906, -.0880)
Severe Hypertension	Stage 1 HTN	1.07633*	.1762	***<0.001***	(.6523, 1.5004)
	Stage 2 HTN	.58929*	.2083	**0.017**	(.0880, 1.0906)

**The mean difference is significant at 0.05*

4.4.5 Correlation and regression analysis of D-DI Level with Independent Variables

In our study, different variables were assessed for possible relationship with plasma D-DI level, and their correlation coefficients and p-values are displayed in **Table 11** and **Figure 6**. Bivariate correlation analysis (**Table 11**) followed by linear regression modeling (**Table 12**) was performed to study the relationship between plasma D-DI level and independent variables. Depending on the nature of variables, Pearson's, point biserial and spearman's rank correlation was performed. Among hypertensive patients, age (r=.285, p=0.004), BMI (r=.214, p=.032), SBP (r=.312, p=.002), DBP (r=.221, p=.027), presence of complication (r_b=.57, p<.001) and severity of HTN (ρ=.66, p<.001) were found to have statistically significant positive correlation with D-DI values. The mean plasma D-DI level was increased with age, SBP (**Figure 6)**, severity of HTN and DBP (not shown in the plot). It was also shown that number of antihypertensive drugs (r=-0.238, p=0.007) and BP control status (r=-0.804, p=0.033) were negatively correlated with D-DI levels. However, no significant correlations existed between D-DI values and sex, smoking status, alcohol consumption, physical activity, family history of HTN or duration of HTN.

Table 11:Correlation between plasma D-dimer level and independent variables

Variables	Correlation coefficient	P-value
Age (year)	.285 [r]	0.004**
Sex	-.071 [rb]	0.591
Smoking status	.095 [ρ]	0.350
Alcohol Consumption	-.146 [rb]	0.267
Physical activity	.047 [rb]	0.722
BMI (kg/m^2)	.214 [r]	0.032 *
Family history of hypertension	-.053 [r]	0.685
Number of antihypertensive drugs	-.238 [r]	0.007**
SBP (mmHg)	.312 [r]	0.002**
DBP (mmHg)	.221 [r]	0.027 *
Duration of HTN (year)	.147 [r]	0.263
Complication	.570 [rb]	<0.001***
Severity of HTN	.660 [ρ]	<0.001***
Controlled BP	-.804 [ρ]	0.033*

******** Correlation is significant at the 0.001 level; ******significant at 0.01; ***** significant at 0.05 level (2-tailed).*
[r]Pearson's correlation coefficient; [rb]Point biserial correlation Coefficient; [ρ]Spearman's correlation coefficient.

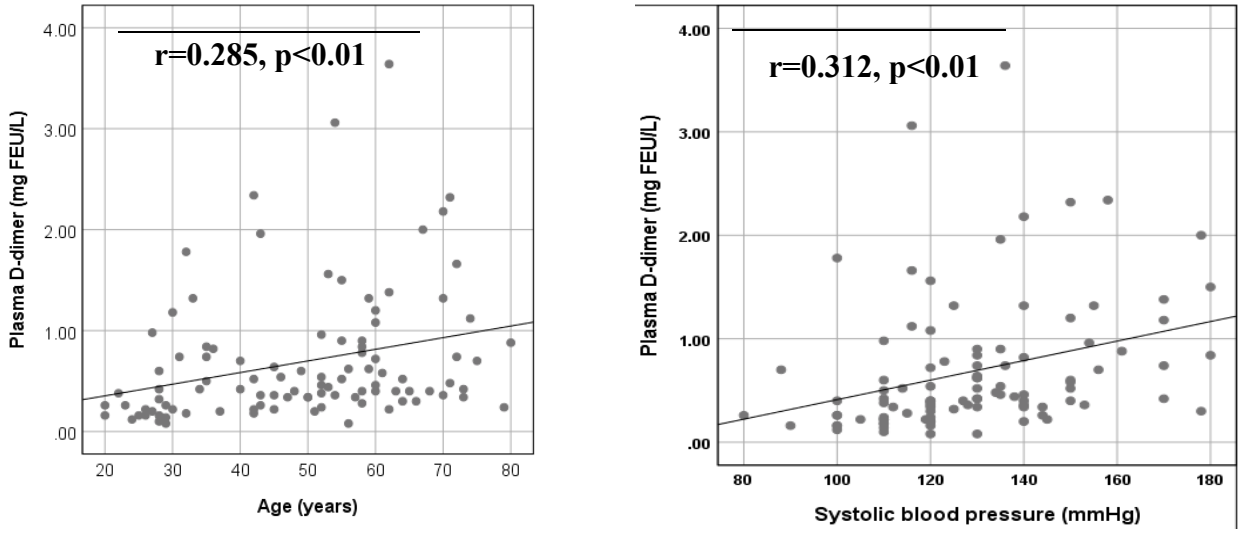

Figure 6: Scatter plot depicting the trend of correlations between D-DI level and Independent variables

As illustrated in **Table 12**, multiple linear regression analysis was performed to quantify the nature of relationships between plasma D-DI level and explanatory variables after adjusting for the effects of other variables. Among all other variables, SBP, severity of HTN, presence of complication and BP control status were found to have independently significant association with plasma D-DI level. Systolic BP was found to be a significant predictors of plasma D-DI level in HTN in which one unit increase in SBP elevates the D-DI level by 0.001(B=0.001, p<0.023). It was also observed that there was a significant change in the level of plasma D-DI across the clinical stages (severity) of HTN. Accordingly, for a one unit increase to a higher-level clinical stage, there was an increase in D-DI level by 0.569 (B=0.569, p<0.001). While all other independent variables are held constant, the presence of complication, such as stroke or heart diseases, significantly increased the plasma D-DI level by factor of 1.048 (B= .048, p < 0.001). Poorly controlled HTN has 0.41 higher D-DI level than well-controlled HTN.

Table 12:Multiple linear regression analysis to see the factors affecting D-DI level in hypertensives.

Variables	Plasma D-dimer (mg FEU/L)			
	B*	**95% CI for B**		**P-value**
		Lower	Upper	
Age, year	.003	-.010	.016	0.636
BMI (kg/m^2)	.024	-.008	.056	0.136
SBP (mmHg)	.001	.008	.013	**0.023**
DBP (mmHg)	.004	-.019	.021	0.736
Presence of complication	1.048	.691	1.405	**<0.001**
No. of antihypertensive drugs	.211	-.015	.438	0.067
Severity of hypertension	.569	.373	.764	**<0.001**
BP control status	.410	.262	.543	**0.020**

**B indicates unstandardized model coefficients to indicate how much the dependent variable varies with an independent variable when all other independent variables are held constant. P-values written in bold are significant at<0.05.*

4.4.6 Factors associated with Hypertension

From multiple binary logistic regression, this study found that age ≥50 years, physical inactivity, BMI≥30kg/m^2 and elevated D-DI level were predictor variables significantly associated with HTN (**Table 13**). However, other variables (sex, smoking, alcohol consumption and family history of HTN) were not significantly associated with HTN after adjusting for confounders. Among all, those who were in 50-59 and 60-80-year age group were, respectively, 1.5 (AOR :1.5, 95%CI: 1.1, 2.9) times and 5.4 (AOR:5.4, 95%CI: 2.1, 7.9) time more likely to develop HTN than those who were in 20-29 years age group. Compared to controls, hypertensive patients were 4.8 (AOR:4.8, 95%CI: 1.9, 5.4) times more likely to be obese, 6.9 (AOR:6.9, 95%CI: 2.7, 17.6) times more likely to have a significantly elevated plasma D-DI levels and 1.9 (AOR:1.9, 95%CI: 1.4, 3.2) more likely to be physically inactive.

Table 13:Binary logistic regression analysis showing factors associated with Hypertension

Variable	**Category**	**n (%)**		**AOR (95%CI)**	**P-value**
		HTN (60)	Control (40)		
Sex	Male	23(38.3)	18(45.0)	0.8 (0.7, 1.7)	0.50
	Female	37(61.7)	22(55.0)	__	__
Age group	20-29	0 (0.0)	18(45.0)	__	__
	30-39	5(8.3)	7(17.5)	1.3(0.9, 2.9)	0.40
	40-49	9(15.0)	7(17.5)	1.0(0.9,3.7)	0.08
	50-59	18(30.0)	7(17.5)	1.5(1.1,2.9)	**0.04**
	60-80	28(46.7)	1(2.5)	5.4(2.1,7.9)	**0.02**
Smoking status	Never	56(93.3)	39 (97.5)	__	__
	Prior or current	6(6.7)	1(2.5)	1.7(1.2, 4.9)	0.243
Alcohol use	Yes	2(3.3)	2(5.0)	0.7 (0.5, 5.1)	0.683
	No	58(96.7)	38(95.0)	__	__
Physical activity	Yes	16(26.7)	12 (30.0)	__	__
	No	44(73.3)	28(70.0)	1.9(1.4, 3.2)	**0.047**
Family history of HTN	Yes	18(30.0)	10 (25.0)	1.2 (0.5, 3.1)	0.664
	No	42(70.0)	30(75.0)	__	__
BMI group	Underweight (<18.5)	3 (5.0)	4(10.0)	__	__
	Normal (18.5-24.9)	23(38.3)	23(57.5)	0.8 (0.7, 1.2)	0.725
	Overweight (25-29.9)	16(26.7)	8(20.0)	1.7 (0.7, 3.2)	0.064
	Obese (≥30kg/m^2)	18(30.0)	5(12.5)	4.8 (1.9,5.4)	**0.007**
D-dimer level	Normal (<0.5mg/l)	22(36.7)	32(80.0)	__	__
	Elevated(≥0.5mg/l)	38(63.3)	8(20.0)	6.9(2.7,17.6)	**<0.001**

P-values written in bold are significant at<0.05

4.5.7 Diagnostic performance of D-dimer levels for disease severity in Hypertension

The diagnostic performance of D-DI for presence of thromboembolic complication in hypertensive patients was further investigated using a receiver operating characteristic (ROC) curve. We found that the area under the curve (AUC), measuring the overall performance of the D-DI test, was 0.869 (95% CI:0.773–0.964) at p<0.001 which indicated AUC is significantly different from 0.5.

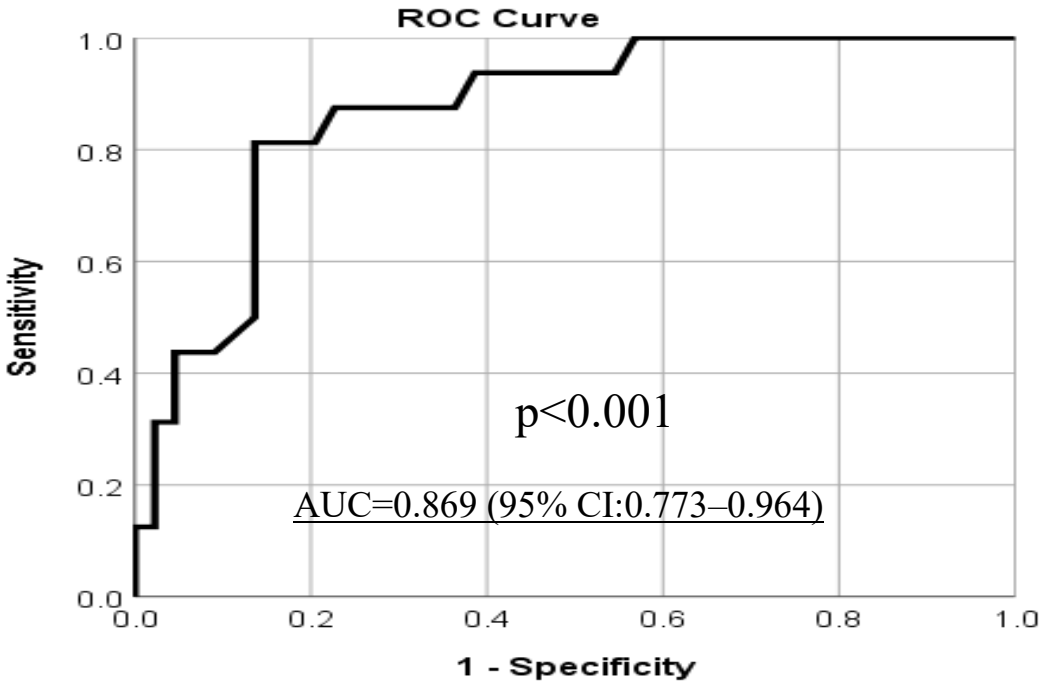

Figure 7:ROC curve analysis for the prediction of complication. AUC indicates the diagnostic power of D-dimer levels

The optimal cut-off value for the D-DI concentration (0.83 mg/l FEU) was selected based on ROC curve analysis. As shown in **Table 14**, the amount of D-DI was determined to be an effective diagnostic marker for severe (complicated) HTN. At a cut-off value of 0.83mg/l, the D-DI concentration had a sensitivity of 87.5%, a specificity of 77.5% with a PPV of 87.5% and NPV of 77.3%. The D-DI test also had an accuracy of 80.0%.

Table 14:The diagnostic power of D-DI in differentiating complicated HTN from uncomplicated

		Actual status(N)						
Cut off value	**Test**	**Complicated**	**Uncomplicated**	**Sensitivity, %**	**Specificity, %**	**PPV, %**	**NPV, %**	**Accuracy, %**
D-DI (0.83mg/l)	**Complicated**	14	10	87.5	77.5	87.5	77.3	80.0
	Uncomplicated	2	34					

N-number of patients; PPV-Positive Predictive Value; NPV-Negative Predictive Value

5. DISCUSSION

The association between HTN and the activation of blood coagulation was elucidated in different literatures. Commonly observed in hypertensive patients, the hypercoagulable state appears to act as an important risk factor for thrombotic complications and may play a role in disease progression (Armas-Herna´ndez *et al.*, 2007). Among the degradation products resulting from the proteolytic actions of plasmin on fibrin, D-dimer is the smallest fibrin degradation by-product, exhibiting unique characteristics. D-DI can be used to estimate the state of activation of the coagulation system, which is elevated by increasing fibrin formation and fibrinolysis (Riley *et al.*, 2016).

This study, therefore, strived to assess plasma D-DI level and its correlation with disease severity among hypertensive patients. We utilized a fully automated quantitative approach for the determination of D-DI level in 60 hypertensive patients and the values were compared with 40 unmatched normal subjects as controls. Our data demonstrated that about 63.3% of hypertensives had excessive concentrations of plasma D-DI level. The study also indicated that plasma D-DI levels significantly increased among hypertensive patient compared to healthy controls. This finding is consistent with a number of studies that have found elevated plasma D-DI levels in essential hypertension and white coat hypertension (WCHT) groups than in healthy controls (Vaziri, 1993;Lip *et al.*, 1997;Catena *et al.*, 2000;Chi, 2002;Coban *et al.*, 2004;Ibrahim and Abdalla, 2014). A comparative cross sectional study has also supported this finding that hypertensive patients tended to show a unbalanced fibrinolytic system and tendency towards a hypercoagulability and a more frequent thrombotic complications as compared to normotensive subjects (Armas-Herna´ndez *et al.*, 2007). Finding of high fibrin D-DI in patients with HTN suggests hypercoagulable state and ongoing intravascular fibrin formation (and possibly early thrombus formation) that plays a role in the pathogenesis of CVDs and complications of HTN. However, this finding was conflicting with another study done in Sudan and a study by Sechi, *et al* who showed that the D-DI level was insignificantly increased in hypertensive patients when compared with a healthy control group (Sechi *et al.*, 2000;Osman and Muddathir, 2013). This discrepancy may probably be related to a difference in sample size and study design.

Based on the finding of this study, age, BMI, DBP, SBP, presence of complication and severity of HTN were found to have statistically significant positive correlation with D-DI values among the hypertensive patients, indicating that higher D-DI levels are associated with higher values of these variables. In contrast, the number of antihypertensive drugs and controlled BP were negatively correlated with D-DI levels, indicating that higher D-DI values were associated with lower values of these variables.

The study results have clearly shown that the plasma D-DI level in patients with HTN was significantly increased with age and BP, elevated level of which was associated with advanced age and high BP. This finding was in agreement with a community-based follow up study done by Pieper *et al* (2000). Another study similarly reported that the levels of plasma D-DI were related to diastolic BP (Coban *et al.*, 2004). The significantly positive correlation between age and D-DI level may be due to age's influence on body function, meaning, 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 plasma D-DI level in patients with HTN was also significantly increased with BMI. This contradicts with another study, showing insignificant correlation of D-DI level and BMI (Kabrhel *et al.*, 2010).

The number of antihypertensive drugs the patient was using and controlled BP were found to have negative correlation with D-DI level. As the number of drugs taken by hypertensives were increased, the D-DI level decreased while the hypertensive patients with poorly controlled BP were found to have significantly higher mean plasma D-DI level. This result agreed with a study that reported antihypertensive drugs; enalapril and terazosin could reduce the level of BP and hence decrease the D-DI level ($P < 0.01$) (Zhang, 2003). This suggests that lowering and controlling BP by appropriate and sufficient number of medications has a paramount importance in decreasing D-DI level and minimizing associated problems.

After adjustment of confounders, through multiple linear regression, SBP, severity of HTN, presence of complication and BP control status were found to be possible predictor variables of D-DI level though the direction of causation was poorly defined which is the inherent property of cross-sectional study design. Unlike DBP, SBP was found to be the significant predictors of plasma D-DI level in HTN in which one unit increase in SBP elevates

the D-DI level by 0.001. This is partially consistent with a report which indicated that high SBP and DBP were found to be independent predictors of plasma D-DI level (Pieper *et al.*, 2000). Poorly controlled HTN has 0.41 higher D-DI level than the well-controlled HTN suggesting controlling BP has a lowering effect on D-DI level. It was observed that, while all other independent variables are held constant, the presence of complication and a one level increase to the higher stage of HTN significantly increased the plasma D-DI level by factor of 1.048 and 0.569 respectively.

Recent study has shown that an activation of the hemostatic system plays a role in development of vascular and organ complication in HTN (Tabak *et al.*, 2009). One of the main pathophysiological aspects of HTN complication is derangements in coagulation profile. This is supported from the observation that satisfactory BP reduction with non-drug intervention and with various classes of antihypertensive drugs does not lead to an equal reduction in heart attacks and strokes which may be due in part to unfavorable effects on the hypercoagulable state in HTN (Lip and Li-Saw-Hee, 1998). In this study, D-DI which shows the activity of fibrinolytic system in coagulation process was used as a marker to evaluate the relationship between abnormal coagulation/fibrinolysis and severity of HTN.

The novel finding of the current study was the association of plasma D-DI with the severity of HTN in which a more severe stage of HTN has a significantly higher D-DI values than the lower stages. The finding was unchanged after the adjustment for confounding variables in which a one level increase to the higher stage of HTN significantly increased the plasma D-DI level by factor of 0.569. This suggests that patients with elevated plasma D-DI level have more severe HTN compared to those hypertensive patients with normal D-DI level though the poor positive predictive value of the test require supportive test to exclude other possible causes of elevated D-DI and to arrive at conclusion. This is in corroboration with previous studies which showed that the level of D-DI increased by progression of HTN to higher or more severe stage (Sechi *et al.*, 2000;Zhang, 2003). A study by Sechi *et al* (2000) showed the relationship between D-DI levels with the severity of target organ damage (TOD) in hypertensive patients. Another study also demonstrated that D-DI is related to different levels of BP, dangerous states and TOD (Zhang, 2003).

D-dimer levels were significantly elevated (by a factor of 1.048) in patients with complicated HTN, such as stroke and heart diseases, compared with those patients without complication while all other independent variables are held constant. These results are consistent with findings from a prior study by Chi *et al* demonstrating that hypertensive patients with left ventricular hypertrophy, left ventricular enlargement, and left atrial enlargement, were detected to have higher levels of D-DI (Chi, 2002). This also got support from Sechi *et al* who reported that higher D-DI levels were independently associated with advanced TOD in hypertensive patients (Sechi *et al.*, 2000). It has been suggested that thrombus formation is involved in HTN progression to complicated type as a result of promoting vessel thrombus occlusion and embolism though further studies with more laboratory investigations and clinical data are needed for logical interpretation and accurate conclusion of the association of an elevated D-DI level and TOD in hypertensive patients. This is an important finding with a benefit for the management of such patients.

The complex interplay between modifiable and non-modifiable factors or conditions, including both environmental and genetic factors, are reported to play a role in the development of primary HTN (Tomson, 2005). In this study, clinical risk factors of HTN were defined based on measuring traditional cardiovascular risk factors such as history of smoking, alcohol consumption, physical activity, family history of HTN and BMI as well as D-DI level.

Our study found that age above 50 years old, physical inactivity, and BMI$\geq$30kg/m^2 were predictor variables significantly associated with being hypertensive for all study participants. This is in accordance with the literature, in which these factors have been intensively studied to contribute to HTN development and even to cardiovascular disorder (Tomson, 2005) and a meta-analysis done in Ethiopia (Kibret and Mesfin, 2015). The age group of 50-59 and 60-80-years were found to develop HTN than in the age group of 20-29 years by a factor of 1.5 and 5.4 times respectively. Those who were obese and doing no exercise were nearly five and two times more likely to be hypertensive, respectively, than their respective references. This is in line with the studies done in Ethiopia (Kibret and Mesfin, 2015;Asresahegn *et al.*, 2017). But, unlike the literatures by Tomson (2005), and Kibret and Mesfin (2015), traditional risk factors such as smoking, alcohol consumption and family history of HTN were not significantly associated with HTN in this study, which could

be due to the difference in study design and sample size. However, this study finding was consistent with a cross sectional study done in Ethiopia (Asresahegn *et al.*, 2017).

Coagulation and fibrinolysis markers have the potential to serve as predictors of disease and disease severity. This is evident from logistic regression analysis of the current study in that apart from age >50 years, BMI$\geq$30kg/m^2 and physical inactivity, elevated D-DI level was an independent risk marker of HTN after controlling for other traditional risk factors. We observed that compared to those with normal D-DI level, those with elevated D-DI level were nearly seven times more likely to develop HTN though this needs further longitudinal or retrospective studies to confirm and arrive at conclusion of D-DI as risk marker.

D-dimer is also predictive of the presence and severity of hypertension-related damage in different organs. D-dimer is a classic marker, which is easy and convenient to test and could be measured in prediction of thrombotic complications associated with hypertension. The ROC curve analysis and the corresponding area under the curve (AUC) in this study showed that plasma D-DI as a biomarker has a predictive ability to discriminate complicated hypertension from uncomplicated one. The level of D-DI was determined to be an effective diagnostic marker for thrombotic complications in hypertension with an AUC of 0.869 (95% CI:0.773–0.964) as well as with a sensitivity and a specificity of 87.5%, and 77.5% respectively at 0.83mg/l FEU cut-off value and an accuracy rate of 80.0%. To the best of our knowledge, ours is the first report from a comparative cross-sectional study demonstrating the diagnostic power of D-DI level in prediction of thrombotic complications in hypertension. This intriguing finding led us to suggest that D-DI measurement can be a useful method to screen for hypertension related TOD or complications resulting from hypercoagulability and can also be useful in identifying patients with higher risk of TOD progression whereas further data will be required to help substantiate the finding.

6. STRENGTH AND LIMITATION OF THE STUDY

6.1 Strength of the study

This study has the strength of revealing pertinent information with respect to several socio demographics, risk factors, clinical and anthropometric parameters claimed to be associated with D-DI level. In addition, the direct measurement (instead of the report from the participant) of anthropometric parameters and BP could be considered as strong side of the study. Moreover, the study involved the sample from both healthy controls and hypertensive patients to clearly compare and show the difference in study variables between the two groups.

6.2 Limitation of the study

Despite the aforementioned strengths, this study is not without drawbacks as described below:

- The high cost of reagents and supplies were limited ourselves to the 100 study participants and only D-DI as marker of coagulation.
- As the study was conducted in only one hospital and the sample size was small, our findings may not well represent all cases and may not be sufficient for generalization.
- Lack of ample previous studies limited the comparison of our study findings with other findings.
- Since it was a cross sectional study design, there is the possibility of residual confounding variables as is observational studies and because the exposure and outcome were simultaneously assessed, and hence generally it couldn't show temporal relation between cause and effect. That is, although the investigators determined an association between exposure and outcome, there is generally no evidence that the exposure caused the outcome. Of course, if the exposure is a characteristic such as sex and the outcome developed over time, the temporal nature of the exposure-outcome association is more plausible; however, for studies in which the exposure is not an inherent trait, but one that developed over time, causality is often unclear.

7. CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

In conclusion, this work suggests that plasma levels of D-DI were higher in hypertensives than the control groups and confirmed the hypercoagulable state among hypertensive patients.

This study also concluded that the severity of HTN were found to have statistically significant positive correlation with D-DI values. Besides, age, BMI, SBP, DBP and presence of complication were found to have statistically significant positive correlation with D-DI values while number of antihypertensive drugs and BP control status were shown to have negative correlation with D-DI levels. High level of D-DI was associated independently with disease severity, complication of HTN, SBP and poorly controlled BP. Patients with severe HTN tend to have higher concentrations of D-DI than those with stage 1 and stage 2 HTN. Besides, controlled BP were found to have lower D-DI level among hypertensive patients.

Apart from age above 50 years, physical inactivity and BMI 30kg/m^2 or greater, elevated D-DI level as independent predictor variables was significantly associated with HTN. Thus, our data suggest that hypertensive subjects, without other cardiovascular risk factors, revealed abnormalities of hemorheological and thrombogenesis, although it was lower than patients with complicated HTN.

Furthermore, plasma D-DI analysis is an inexpensive, noninvasive and simple method that may be useful as a guide in predicting the severity of the disease in patients with HTN. Our data demonstrate that plasma D-DI have been shown to have very good predictive power for thromboembolic diseases or complications like heart diseases and stroke, and this biomarker could serve as a valuable predictor for complication development in HTN as evidenced by some of hypertensive patients (36.7%) having marked elevated concentrations of D-DI without any evidence of thromboembolic complications related to HTN with the currently available diagnostic modalities .

7.2 Recommendations

Based on the findings of our study, the following recommendations have been forwarded:

- 🕮 There should be screening and timely evaluation of hypertensive patients for disease severity and thromboembolic complications. It is required to elucidate the mechanisms through which HTN is associated with target organ damage or complication. But elucidating these underpinnings is an enormous task. We strongly believe that our study is an attempt at unlocking one such portion of this systemic puzzle that still requires tremendous work in the future.
- 🕮 Clinical care by managing hypertensive patients to control BP level using appropriate type, number and combination of drugs whenever indicated lowers D-DI level and prevent HTN complications though further interventional studies need to be conducted to determine whether better control of HTN could really prevent complications.
- 🕮 Focusing attention on traditional cardiovascular risk factors such as physical inactivity and obesity, might be the most appropriate method to manage HTN related morbidity and complications.
- 🕮 This study was cross sectional and we strongly believe that further studies need be done on larger scale using more robust case control and cohort studies.
    - ☞ For more accurate evaluation of plasma D-DI levels in different stages of HTN.
    - ☞ To establish the causality of the association between the severity of HTN and D-DI level and their diagnostic implications on prediction of complication associated with of HTN.
    - ☞ To see how D-DI detect the complications accurately in early stage and also to compare the accuracy with other diagnostic modalities, biochemical and imaging tests that are currently used to specifically detect different types of HTN complications.
    - ☞ To show the effect of antihypertensive drugs on plasma D-DI level.

8. REFERENCES

Adam, S. S., Key, N. S. & Greenberg, C. S. 2009. D-dimer antigen: current concepts and future prospects. *Blood,* 113**,** 2878-87.

Alemu, G., Natesan, G. & Menakath, M. 2017. Evaluation of Lipid Profiles and Hematological Parameters in Hypertensive Patients: A Cross Sectional Study at Debre Markos Referral Hospital, North West Ethiopi.

Alfonso, L. C., Edelson, G.W., & Sowers, J.R. 1997. Metabolic abnormalities in hypertension. *Curr Opin Nephrol Hypertens.,* 6**,** 219–223.

Armas-Herna´Ndez, M. a., Jose´ , Rafael Herna´Ndez-Herna´Ndez, Marı´a Cristina Armas-Padilla, M., 1 Beatriz Sosa-Canache, , Rosalba Cammarata, Beatriz Pacheco, Jaime Guerrero-Pajuelo, Zafar H. Israili, a. & Manuel Valasco 2007. Fibrinolytic System in Normotensive Subjects and Hypertensive Patients. *American Journal of Therapeutics* 14**,** 177–182

Asresahegn, H., Tadesse, F. & Beyene, E. 2017. Prevalence and associated factors of hypertension among adults in Ethiopia: a community based cross-sectional study. *BMC research notes,* 10**,** 629.

Awoke, A., Awoke, T., Alemu, S. & Megabiaw, B. 2012. Prevalence and associated factors of hypertension among adults in Gondar, Northwest Ethiopia: a community based cross-sectional study. *BMC cardiovascular disorders,* 12**,** 113.

Beaglehole , R., Epping-Jordan, J., Patel, V., Chopra, M., Ebrahim, S., Kidd, M. & Haines, A. 2008. Improving the prevention and management of chronic disease in low-income and middle income countries: A priority for primary health care. *Lancet,* 372**,** 940-9.

Beevers, G., Lip, G. Y. & O'brien, E. 2001. The pathophysiology of hypertension. *Bmj,* 322**,** 912-916.

Blann, A. D., Naqvi, T., Waite, M. & Mccollum, C. N. 1993. Von Willebrand factor and endothelial damage in essential hypertension. *J Hum Hypertens.,* 7**,** 107–11.

Bygbjerg, I. C. 2012. Double burden of noncommunicable and infectious diseases in developing countries. *Science,* 337**,** 1499–501.

Carey, R. M. & Whelton, P. K. 2018. Prevention, detection, evaluation, and management of high blood pressure in adults: synopsis of the 2017 American College of Cardiology/American Heart Association hypertension guideline. *Annals of internal medicine,* 168**,** 351-358.

Catena, C., Zingaro, L., Casaccio, D. & Sechi, L. A. 2000. Abnormalities of coagulation in hypertensive patients with reduced creatinine clearance. *The American journal of medicine,* 109**,** 556-561.

Chabloz, P., Reber, G., Boehlen, F., Hohlfeld, P. & De Moerloose, P. 2001. TAFI antigen and D-dimer levels during normal pregnancy and at delivery. *Br J Haematol* 115**,** 150-2.

Chi, D. S., et al. 2002. Coagulative and fibrinolytic changes in patients with essential hypertension and the effect on sustainedrelease nifedipine. *Di Yi Jun Yi Da Xue Xue Bao* 22**,** 168–170.

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., Jones, D. W., Materson, B. J., Oparil, S., Wright, J. T. & Roccella, E. J. 2003. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 42**,** 1206–52.

Cimmino, G., Fischetti, S. & Golino, P. 2017. The Two Faces of Thrombosis: Coagulation Cascade and Platelet Aggregation. *Are Platelets the Main Therapeutic Target***,** 2.

Coban, E., Özdoğan, M. & Akcit, F. 2004. Levels of plasma fibrinogen and D-dimer in subjects with white-coat hypertension. *Journal of human hypertension,* 18**,** 291.

Coffey, C. S., Asselbergs, F. W., Hebert, P. R., Hillege, H. L., Moore, Q., Li, J. H. & Van Gilst, W. H. 2011. The association of the metabolic syndrome with PAI-1 and t-PA levels,. *Cardiol. Res. Pract,* 2011**,** 541-467.

De Lorenzo, A., Soldati, L., Sarlo, F., Calvani, M., Di Lorenzo, N. & Di Renzo, L. 2016. New obesity classification criteria as a tool for bariatric surgery indication. *World journal of gastroenterology,* 22**,** 681.

Dell'omo, G., De Negri, F. & Carmassi, F., et al. 2003. Reactive hyperemia and tissue-type plasminogen activator release in hypertensive men. *Blood Coagul Fibrinolysis,* 14**,** 19–25.

Dreisbach, A. W. 2013. Epidemiology of Hypertension. *Supplement to JAPI,* 61**,** 12–13.

Elliott, W. J. 2006. Clinical features in the management of selected hypertensive emergencies. *Progress in Cardiovascular Diseases,* 48**,** 316-325.

Foe¨X, P. & Sear, J. W. 2004. Hypertension: pathophysiology and treatment. *OXFORD JURNALS* 4**,** 71-5.

Fowkes, F. G. R., Aboyans, V., Fowkes, F. J., Mcdermott, M. M., Sampson, U. K. & Criqui, M. H. 2017. Peripheral artery disease: epidemiology and global perspectives. *Nature Reviews Cardiology,* 14**,** 156.

Fowkes, F. G. R., Lowe, G.D.O, Housley, E., Rattray, A., Rumley, A., Elton, R.A., MacGregor, I.R. and Dawes, J. 1993. Cross-linked fibrin degradation products, progression of peripheral arterial disease, and risk of coronary heart disease. 342 84–86.

Furie, B. a. F., B.C. 2005. Thrombus formation in vivo. *J. Clin. Invest.* , 115**,** 3355–62.

Gaffney, P. J. 1973. Subunit relationships between fibrinogen and fibrin degradation products. *Thromb. Res.*, 2**,** 201–217.

Gaffney, P. J. 2001. Fibrin Degradation Products. *Annals of the New York Academy of Sciences,* 936**,** 594-610.

Gifford, R. 1991. Management of hypertensive crises. *Jama,* 266**,** 829-835.

Gleerup, G., and Winther, K. 1991. Decreased fibrinolytic activity and increased platelet function in hypertension. Possible influence of calcium antagonism. *Am J Hypertens.,* 4**,** 168S–171S.

Goldstein, L. B., Adams, R., Alberts, M. J., Appel, L. J., Brass, L. M., Bushnell, C. D., Culebras, A., Degraba, T. J., Gorelick, P. B. & Guyton, J. R. 2006. Primary prevention of ischemic stroke: A guideline from the American heart association/American stroke association stroke council. *Stroke,* 37**,** 1583-1633.

Hager, K., and Platt, D. 2009. Fibrin Degeneration Product Concentrations (D-Dimers) in the Course of Ageing. *Gerontology* 41**,** 159–165

Harmening, M. D. 2009. *Clinical Hematology and Fundamentals of Hemostasis.* , Philadelphia, F.A. Davis Company.

Harper, P. L., Theakston, E., Ahmed, J. & Ockelford, P. 2007. D-dimer concentration increases with age reducing the clinical value of the D-dimer assay in the elderly. *Internal Medicine Journal* 37**,** 607–613.

Ibrahim, H. M. & Abdalla, M. H. 2014. D-Dimer Levels in Sudanese Hypertensive Patients. *American Journal of Medicine and Medical Sciences,* 4**,** 92-95.

Ioannidis, J. P. 2018. Diagnosis and treatment of hypertension in the 2017 ACC/AHA guidelines and in the real world. *Jama,* 319**,** 115-116.

James, P. A., Oparil, S., Carter, B. L., Cushman, W. C., Dennison-Himmelfarb, C., Handler, J., Lackland, D. T., Lefevre, M. L., Mackenzie, T. D. & Ogedegbe, O. 2014. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *Jama,* 311**,** 507-520.

Jepson, R. E. 2011. Feline systemic hypertension: classification and pathogenesis. *Journal of Feline Medicine & Surgery,* 13**,** 25-34.

Juhan-Vague, I. & Alessi, M. C. 1993. Plasminogen activator inhibitor 1 and atherothrombosis. *Thromb Haemost,* 70**,** 38–143.

Kabrhel, C., Mark Courtney, D., Camargo Jr, C. A., Plewa, M. C., Nordenholz, K. E., Moore, C. L., Richman, P. B., Smithline, H. A., Beam, D. M. & Kline, J. A. 2010. Factors associated with positive D-dimer results in patients evaluated for pulmonary embolism. *Academic emergency medicine,* 17**,** 589-597.

Kearney, P. M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P.K., and He. J. 2005. Global burden of hypertension: analysis of worldwide data. *Lancet,* 365**,** 217-23

Kibret, K. T. & Mesfin, Y. M. 2015. Prevalence of hypertension in Ethiopia: a systematic meta-analysis. *Public Health Reviews,* 36**,** 14.

Lee, A. J. 1997a. The role of rheological and haemostatic factors in hypertension. *Journal of human hypertension,* 11**,** 767.

Lee, A. J. 1997b. The role of rheology and haemostatic factors in hypertension. *J Hum Hypertens.* , 11**,** 767–776.

Li-Saw-Hee, F. L., Beevers, D. G. & Lip, G. Y. 2001. Effect of antihypertensive therapy using enalapril or losartan on haemostatic markers in essential hypertension: a pilot prospective randomised double-blind parallel group trial. *International journal of cardiology,* 78**,** 241-246.

Lillicrap, D., Key, N., Michael Makris & O'shaughnessy, D. 2009. Practical Hemostasis and Thrombosis. *Wiley-Blackwell***,** pp. 1–5.

Lim, S. S., Vos, T., Flaxman, A.D., Danaei, G., Shibuya, K., Adair-Rohani ,H., et al 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010. *The Lancet,* 380**,** 2224-60.

Lind, L. & Lithell, H. 1993. Decreased peripheral blood flow in the pathogenesis of the metabolic syndrome comprising hypertension, hyperlipidemia and hyperinsulinemia. *Am Heart J,* 125**,** 1494–497.

Lip, G. Y., & Lowe, G.D. 1995. Fibrin D-dimer: a useful clinical marker of thrombogenesis. *Clin Sci* 89**,** 205–14.

Lip, G. Y. & Blann, A. D. 2000. Does Hypertension Confer a Prothrombotic State?: Virchow's Triad Revisited. Am Heart Assoc.

Lip, G. Y. & Li-Saw-Hee, F. L. 1998. Does hypertension confer a hypercoagulable state? *Journal of hypertension,* 16**,** 913-916.

Lip, Y. H., Blann, A. D., Jone, A. F., Dphil, Lip, P. L., Frcsed & Beevers, D. G. 1997. Relation of Endothelium, Thrombogenesis, and Hemorheology in Systemic Hypertension to Ethnicity and Left Ventricular Hypertrophy. *Am J Cardiol* 80**,** 1566–157.

Lottermoser , K., Weisser, B. & Hertfelder, H. J., et al. 1998. Antihypertensive drug treatment and fibrinolytic function. *Am J Hypertens.,* 11**,** 378–384.

Lowe, G. D. 2005. Fibrin D-dimer and cardiovascular risk. *Semin Vasc Med.,* 5**,** 387-398.

Lowe, G. D. O., Rumley, A., Yarnell, J.W.G., Sweetnam, P.M., &Thomas, H.F. 1995. Fibrin D-dimer, von Willebrand factor and tissue plasminogen activator antigens are predictors of major ischaemic heart disease: the Caerphilly study. *Blood Coag Fibrin,* 6**,** 156–157.

Macmahon, S., Peto, R., Cutler, R., Collins, R., Sorlie, P., Meaton, J. et al. 1990. Blood pressure, stroke and coronary heart disease. Part 1. Prolonged differences in blood pressure: prospective observational studies corrected for regression dilution bias. *Lancet* 335**,** 765–74.

Malani, P. N. 2012. *Harrison's principles of internal medicine*.

Mannucci, P. M. & Giangrande , P. L. 1992. Detection of prethrombotic state due to procoagulant imbalance. *Eur J Haematol.,* 48**,** 65– 69.

Mitchell, R. N., Kumar, V., Abbas, A.K., & Fausto, N. (ed.) 2006. *Pathologic Basis of Disease.,* Philadelphia: Saunnders Elsevier.

Muela, H. C., Costa-Hong, V. A., Yassuda, M. S., Moraes, N. C., Memória, C. M., Machado, M. F., Macedo, T. A., Shu, E. B., Massaro, A. R. & Nitrini, R. 2017. Hypertension severity is associated with impaired cognitive performance. *Journal of the American Heart Association,* 6**,** e004579.

Muntner, P., Carey, R. M., Gidding, S., Jones, D. W., Taler, S. J., Wright, J. T. & Jr, e. a. 2017. Potential U.S. population impact of the 2017 American College of Cardiology/American Heart Association high blood pressure guideline. *Circulation*.

Nwankwo, T., Yoon, S., Burt, V. & Gu, Q. 2013. Hypertension among adults in the United States: national health and nutrition examination survey, 2011–2012.

Olson, J. D. 2015. D-dimer: an overview of hemostasis and fibrinolysis, assays, and clinical applications. *Advances in clinical chemistry.* Elsevier.

Osman, S. S. A. & Muddathir, A. R. M. 2013. Measurement of Plasma fibrinogen and D-dimer levels in Sudanese Hypertensive patients. *American journal of research communication,* 1**,** 360-367.

Pickering, T. G., Coats, A. & Mallion, J. M., et al. 1999. Blood Pressure Monitoring. Task Force V: White coat hypertension. *Blood Press Monit :,* 4**,** 333-41. .

Pieper, c. f. K., Murali, K., Rao, M. S., Currie, T. B., Harris & Cohen, H. J. 2000. Age, Functional Status, and Racial Differences in Plasma D-Dimer Levels in Community-Dwelling Elderly Persons. *Journal of Gerontology:MEDICAL SCIENCES,* Vol. 55A**,** M649–M657.

Poli, K. A., Tofler, G.H., Larson, & M.G., et al. 2000. Association of blood pressure with fibrinolytic potential in the Framingham Offspring Population. Circulation 101**,** 264–269.

Ridker, P. M., Hennekens, C. H., Cerskus, A. & Stampfer, M. J. 1994. Plasma concentrations of cross-linked fibrin degradation product (D-dimer) and the risk of future myocardial infarction among apparently healthy men. . *Circulation,* 90**,** 2236– 2240. .

Ridker, P. M. e. a. 1994. Prospective study of endogenous tissue plasmaingen activator and risk of stroke. *Lancet,* 343**,** 940–943.

Riley, R. S., Gilbert, A. R., Dalton, J. B., Pai, S. & Mcpherson, R. A. 2016. Widely used types and clinical applications of D-dimer assay. *Laboratory medicine,* 47**,** 90-102.

Rocha, E., & Paramo, J.A. 1994. The relationships between impaired fibrinolysis and coronary heart disease: a role for PAI-1. *Fibrinolysis,* 8**,** 284–303.

Rowbotham, B. J., Whitaker, A.N., Harrison, J., Murtaugh, P., & Reasbeck, P., Bowie, E.J. 1992. Measurement of cross-linked fibrin derivatives in patients undergoing abdominal surgery: use in the diagnosis of postoperative venous thrombosis. *Blood Coag Fibrin* 3**,** 25–31.

Ruberg, F. L., & Loscalzo, J. 2002. Prothrombotic determinants of coronary atherothrombosis. *Vasc Med.,* 7**,** 289–299.

Sechi, L. A., Zingaro, L., Catena, C., Casaccio, D. & De Marchi, S. 2000. Relationship of fibrinogen levels and hemostatic abnormalities with organ damage in hypertension. *Hypertension,* 36**,** 978–85.

Smith, F. B., Lee, A.J., Fowkes, F.G., Price, J.F., Rumley, A., & Lowe, G.D. 1997. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh Artery Study. *Arterioscler Thromb Vasc Biol* 17**,** 3321-5.

Tabak, O., Gelisgen, R., Uzun, H., Kalender, B., Balci, H., Curgunlu, A., Simsek, G. & Karter, Y. 2009. Hypertension and hemostatic/fibrinolytic balance disorders. *Clinical & Investigative Medicine,* 32**,** 285-292.

Tambe, D., Phadke, A., Kharche, J. & Joshi, A. 2010. Correlation of blood pressure with Body Mass Index (BMI) and Waist to Hip Ratio (WHR) in middle aged men. *Internet Journal of Medical Update-EJOURNAL,* 5.

Thom, T. J., Epstein, F.H., & Felman ,F.F. 1992. Total mortality and morbidity from heart diseases, cancer and stroke from 1950-1987 in 27 countries. *NIH Pub***,** 92-3088.

Thompson, S., Kienast, J., Pyke, S., Haverkate, F. & Van De Loo, J. 1995. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N Engl J Med,* 332**,** 635-641.

Tomson, J., & Lip, G. Y. 2005. Blood pressure demographics: nature or nurture..... genes or environment? *BMC medicine,* 3.

Vaziri, N. D., et al. 1993. Coagulation and inhibitory and fibrinolytic proteins in essential hypertension. *J Am Soc Nephrol.,* 4**,** 222–228.

Vaziri, N. D., Gonzales, E. C., Wang, J. & S, S. 1994. Blood coagulation, fibrinolytic, and inhibitory proteins in end-stage renal disease: effect of hemodialysis. *Am J Kidney Dis.* , 23**,** 828– 835.

Versteeg, H. H., Heemskerk, J.W., Levi, M., & Reitsma, P.H. 2013. New fundamentals in hemostasis *Physiol Rev,* 93**,** 327-358.

Wall, U., Jern, C., Bergbrant, A., et al. 1995. Enhanced levels of tissue-type plasminogen activator in borderline hypertension. *Hypertension* 26**,** 796–800.

Wells, P. S., Anderson, D. R., Rodger, M., Forgie, M., Kearon, C., & & Dreyer, J. 2003. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. *N Engl J Med.* , 349**,** 1227-35.

WHO 2011. Non communicable diseases country profiles.

WHO 2013. Global Health Repository. Raised blood pressure: situation and trends.

WHO 2018. Noncommunicable diseases country profiles 2018.

Wubaye, W. D., Yeweyenhareg, F., Dejuma, Y. & Tedla, K. 2016. Ethiopian National Guideline on Major NCDs 2016.

Zhang, W. R., Sun, M. , & Luo, J.K. 2003. Serum nitric oxide and D-dimer before and after administering antihypertensive drugs in essential hypertension. *life-sciences literature,* 28 382-384.

9. ANNEXES

Annex I: English Version Information Sheet

Addis Ababa University, School of Medicine, Department of Medical Biochemistry

Research project: Assessment of plasma D-dimer level and its correlation with disease severity among hypertensive patients at Yikatit 12 Hospital.

Principal Investigator: Endeshaw Chekol (BSc)

Advisors: 1-Dr. Solomon Genet 2-Dr. Menakath Menon

Sponsoring organization: Addis Ababa University

Introduction: Good morning/afternoon dear participant! My name is _____. I am working as a data collector for the study conducted in Yikatit 12 Hospital by Endeshaw Chekol who is studying for MSc degree in Medical Biochemistry at Addis Ababa University, College of Health Sciences. I kindly request you to lend me your attention to explain about the study and being you selected as the study participant.

Study Objective: The purpose of this study is to evaluate plasma D-dimer level and its correlation with disease severity among hypertensive patients.

Procedures: If you agree to take part in this study, you will be given the consent form to sign, and interviewed by health professional to assess whether you qualify to participate in the study or not. If you are fit for the study, the data collector will ask some questions which are important for the study like socio-demographic. Physical measurements like weight, height, and blood pressure will be taken. 4mL of blood sample will be also collected for laboratory examination of D-dimer.

Possible risks: The study has no health risk except minimum pain associated with blood with drawl procedure. This is just the result of the routine work which experienced by all the individuals that will involve in the research by experienced health practitioner.

Possible Benefit: Any incentive to you during participation in this study will not be given as compensation. But you will have the chance to know your general health status from the project without any payment because the cost will be covered by the project. In addition, the result of the study will be beneficial for the determining the relation of D-dimer level with the

severity of hypertension. Hence, you are indirectly benefiting other patients and the society in this regard.

Confidentiality: The information you provide for us will be confidential and only used for research purpose. There will be no information that will identify you in particular. The findings of the study will be general for the study area and will not reflect anything particular of individual persons. The questionnaire will be coded to exclude showing names. No reference will be made in oral or written reports that could link participants to the study.

Rights: To protect the participant from any risk and discomfort which may result due to the procedure of the study, we have written ethical clearance and cooperation letter obtained from Addis Ababa University College of Health Sciences, Department of Biochemistry. Participation for this study is fully voluntary. You have the right to declare to participate or not in the study. If you decide to participate, you have the right to with draw from the study at any time.

At the end, if you faced any problem related to the study or if you have any question about the study, you can contact me (the investigator) via phone: 0905525335/0928428133 or by using email address: endeshawchekole@gmail.com.

Annex II: English Version Consent form

I volunteer to participate in a research project conducted by Endeshaw Chekol from Addis Ababa University. I understand that the project is designed to gather information about investigation of D-dimer in hypertensive patients in Yikatit 12 Hospital, I will be one of participants being selected for this research. I understand that I will not be paid for my participation. I may withdraw and discontinue participation from the study at any time without penalty. I understand that the researcher will not identify me by name in any reports using information obtained from this interview and that my confidentiality as a participant in this study will remain secure. Subsequent uses of records and data will be subject to standard data use policies which protect the anonymity of individuals and institutions.

I have been given a copy of this consent form and I have clearly understood the explanation provided to me. So, I hereby approve my consent with my signature to take part in the study.

_____	_____	_____
Participant's code number	Date	Signature
_____	_____	_____
Data Collector's name	Date	Signature

Annex III: English version questionnaires

Subject code number: _____ Card number_____

Instruction: Dear data collectors, you are kindly requested to include all volunteer hypertensive patients and healthy controls who don't have diseases or conditions such as thromboembolism (DVT and PE), Disseminated intravascular coagulation (DIC), recent surgery or trauma (last 3months), pregnancy, early age (<20), advanced age (>80years), known history diabetes mellitus, known history renal failure, liver disease, anticoagulatory (heparin, warfarin) or thrombolytic (streptokinase or urokinase) treatment either by history from the patients or by reviewing patients card. And please choose and encircle for closed ended questions and fill the provided blank space for open ended questions.

Part I: Socio demographic variables		
S.No.	**Question**	**Response**
101	Age of participant	_____year
102	Sex of participant	1. Male 2. Female
103	Marital status	1. Single 2. Married 3. Divorced 4. Widowed
104	Educational status	1. Illiterate 2. Primary school 3. Secondary school 4. College/university
105	Occupation	1. Housewife 2. Merchant 3. Farmer 4. Government employee 5. Private employee 6. Student 7. Daily laborer
106	Residence	1. Rural 2. Urban

Part II: Risk assessing questions		
201	Smoking*	1.NO 2. Prior smoker 2. Current smoker
***Current smoker:** Those who have smoked > 100 cigarettes in their life time and smoked in the last 28 days. **Prior smoker:** Those who have smoked >100 cigarettes in their life time but not smoked in the last 28 days. **Non-smoker:** Those who haven't smoked >100 cigarettes in their life time and don't currently smoke.		
202	Alcohol use (≥2 drinks per day for women or ≥3 drinks per day for men)	1. Yes 2. NO
203	Regular physical exercise (≥30 min of moderate exercise (brisk walking, cycling) per day for ≥5days per week)	1. Yes 2. NO
205	Family history of hypertension	1. Yes 2. NO
Part III: Clinical Condition		
301	Blood pressure (average BP of 3 readings)	_____ mmHg
302	Duration of hypertension	_____ years
303	Use antihypertensive drug?	1. Yes 2. No
304	If Q303 is "yes" mention the antihypertensive drug the patient using? (specify whether drugs were fixed drug combination or not)	_____
305	If Q303 is "yes" write the number of antihypertensive drug(s) the patient using? (through counting by the data collector)	_____ (write the number here)
306	Complication present**	1.Yes 2. N0
****encircle** 'Yes' in Q306 if the patient has a thromboembolic complication (stroke, heart disease (myocardial infarction, left ventricular hypertrophy, arrythmia or CAD), secondary renal failure, or PAD) developed as a result of hypertension as per the documentation of the patient's card.		
307	If Q306 is "yes" mention the complication (by reviewing patient's card or historically)	_____
308	Stage of HTN (filled by the data collectors)	1. Stage 1 2. Stage 2 3 Severe HTN
Part IV: Anthropometric measurement		
401	Weight	_____ Kilo gram
402	Height	_____meter
403	Body Mass Index (BMI)	_____ kg/m^2
Part V: Biochemical measurement		
501	D-dimer level	_____mg/l FEU

Annex IV: *ለማርኛ ለተሳታፊዎች ስለጥናቱ መረጃ*

ጤና ይስጥልኝ! እኔ_____እባላለሁ፡፡በአሁኑ ሰአት በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ ሕክምና ት/ቤት በባዮኬሚስትሪ ት/ክፍል በማስተርስ ድግሪ የሚያጠናው አቶ እንዳሻዉ ቸኮል ለሚያደርገው የመመረቂያ ጥናት በመረጃ ሰብሳቢነት እየሰራሁ እገኛለሁ፡፡ እናም እርስዎ በዚህ የመመረቂያ ጥናት ላይ እዲሳተፉ ተመርጠዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

የጥናቱ ርዕስ፡- በደም ግፊት ህመምተኞች ደም ዉስጥ ያለውን ዲ-ዳይምር የተባለውን ንጥረ ነገር በመመርመር እና ምንም በሽታ ከለለባቸው ሰዎች ጋር ማዎዳደር እንዲሁም የዲ-ዳይምር መጠን ከደም ግፊት ደረጃ ጋር ያለዉን ዝምድና ላይ የሚደረግ ምርምር ነው፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት: በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል አርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት፡፡

በዚህ ጥናት መሳተፍ ሊያስከትላቻው የሚችሉ ቸግሮች: ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም፡፡ ነገር ግን ደም ሲወሰድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል፡፡ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም፡፡

በዚህ ጥናት መሳተፍ ሊገኙ የሚችሉ ጥቅሞች: ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኝው ውጤት ግን ተጠቃሚ ነዎት፡፡ የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎን የደም ግፊት እና ሊያስከትላቸው የሚችለውን ተዛማጅ በሽታዎች ቀድሞ ለማወቅና ለመከታተል ከፍተኛ ጥቅም ይኖረዋል፡፡

የመረጃ በሚሰጥር አጠባበቅ ሁኔታ: ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ የጥናቱ ውጤት የተሳታፊዎችን አጠቃላይ ሁኔታ እንጂ የአንድን ግለሰብ ምንም ነገር አያንጸባርቅም።የተሳታፉዎችን ስም ላለማሳየት የራሳችንን መለያ ቁጥር የምንጠቀም ይሆናል፡፡ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው፡፡ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ::

በዚህ ጥናት ላለመሳተፍ ያለዎት መብት: በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት

የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ::ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት::የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ:: ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለማወቅ ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን::

ጥያቄ ካልዎት ወይም ችግር ካጋጠመዎት: ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ችግር ከገጠምዎት ወይምጥያቄ ካለዎት በስልክ ቁጥር፡ 0905525335/0928428133 ወይም በኢ-ሜይል: endeshawchekole@gmail.com፤ የሚለውን አድራሻ ይጠቀሙ::

Annex V: አማርኛ የስምምነት ማረጋገጫ ቅጽ

የዚህ ጥናት መሰረታዊ ዓላማ እና ሌሎች መረጃን በሚገባ ተገንዝቢያለሁ:: ጥናቱ በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል በሚገኙ የደም ግፊት ታማሚዎች ላይ በሚከሰተን ዲ-ዳይመር ለመለካት እንደሆነ በሚገባ ተገንዝቢያለሁ:: ተሳትፎዬ በፍቃደኝነት ላይ ብቻ የተመረኮዘ እንደሆነም ተረድቻለሁ:: ማንኛዉም ሰብዓዊም ሆነ ህጋዊ መብቴ ሳይነካ ከጥናቱ ራሴን ማግለል እንደምችልም እንደሆነ:: ስለ ጥናቱ ዝርዝር ጉዳይ በግልፅ ከተረዳሁት ባሻገር በተጨማሪ ማብራሪያ ብፈልግ መጠየቅ እንደምችልም አዉቄያለሁ:: የጥናቱም ባለቤት የዚህ የጥናቱ መረጃ ይፋ የሚሆነው ለእኔ ብቻ እንደሆነ እና ስለሚወሰደው ማንኛውም መረጃዎች ሆነ የጥናት ውጤት ለማሰራጨት በስም ሳይሆን በሚስጥር(ኮድ) እንደሆነም ተረድቻለሁ::

በመሆኑም በፈቃዴ የዚህ ጥናት አካል እንድሆን ስፈልግ የምጠብቅብኝን ሁሉ ለሚድረግ በመወሰን መሆኑን በፊርማዬ አረጋግጣለሁ::

_____	_____	_____
የታሳታፊ የምስጥር ቁጥር	ቀን	ፊርማ
_____	_____	_____
የመረጃ ሰብሳቢው ስም	ቀን	ፊርማ

Annex VI: አማርኛ መጠይቅ

የሚስጥር ቁጥር: _____ *ካርድ ቁጥር*_____

<u>አቅጣጫ</u>: ለምርጫ ጥያቄዎች መልሱን አክብቡ፤ለፃፍ ጥያቄዎች ደግሞ ፊትለፊት ካለው ክፍት ቦታ ላይ መልሱን ይፃፉ፡፡

ክፍል 1: ማህበራዊና ዲሞግራፊያዊ መጠቆች		
ተ.ቁ	**ጥያቄ**	**መልስ**
101	ዕድሜ	_____(በዓመት)
102	ፆታ	1.ወንድ 2. ሴት
103	የጋብቻ ሁኔታ	1. ያላገባ 2.ያገባ 3. የፈታ/ች 4. ባለቤቷ/ቱ በህይወት የሌለ
104	የትምህርት ደረጃ	1. ያልተማረ 2. የመጀመሪያ ደረጃ 3. ሁለተኛ ደረጃ 4.ኮሌጅ/ዩኒቨርስቲ
105	የስራ ሁኔታ	1. የቤት እመቤት 2. አርሶ አደር 3. ነጋዴ 4. የመንግስት ሰራተኛ 5. የግል መስሪያ ቤት ሰራተኛ 6. ሌላ _____ (ካለ ጥቀስ)
106	የመኖሪያ ቦታ	1. ከተማ 2. ገጠር
ክፍል 2: በሽታ አጋላጭ ከሆኑ ነገሮች ጋር ተያያዥነት ያላቸው ጥያቄዎች		
201	ሲጋራ ያጨሳሉ	1. አዎ 2. የለም
202	አልኮል ይጠጣሉ	1. አዎ 2. የለም
203	መደበኛ የአካል ብቃት እንቅስቃሴ ያደርጋሉ	1. አዎ 2. የለም
205	የደም ግፊት ያለበት ዘመድ አለዎት	1. አዎ 2. የለም
ክፍል 3: ከህክምና ጋር ተያያዥ ጥያቄዎች		
301	የደም ግፊት መጠን	_____ሚሊ ሜትር ሜርኩሪ

302	የደም ግፊትዎ ከታወቀ ስንት ጊዜ ሆነዎት	_____ ዓመት
303	የደም ግፊት መድሃኒት እየወሰዱ ነው	1. አዎ 2. የለም
304	ለተ.ቁ 302 "አዎ" ከሆነ መልሱ፤ የትኛዉን መድሃኒት ነዉ እየወሰዱ ያሉ;	_____
305	ለተ.ቁ 302 "አዎ" ከሆነ መልሱ፤ ስንት የደም ግፊት መድሃኒት ነዉ እየወሰዱ ያሉ (በመረጃ ሰብሳቢው የሚሞላ)	_____
306	ከደም ግፊቱ ጋር የተያያዘ መወሳሰብ አለ	1. አዎ 2. የለም
307	ለተ.ቁ 306 "አዎ" ከሆነ መልሱ፤ የመወሳሰብ ችግሩን ይግለፁ	_____
308	የግፊቱ ደረጃ	1. ደረጃ 1 2. ደረጃ 2 3. ከባድ ግፊት
ክፍል 4: የአካል ልኬት		
401	ክብደት	_____ኪሎ ግራም
402	ቁመት	_____ሜትር
403	የሰውነት ክብደት ልኬት	_____(በኪ.ግ/ሜ^2
ክፍል 5: የላብራቶሪ ምርመራ ዉጤት		
501	የ ዲ-ዳይመር መጠን(በሚ.ግ/ሊ)	_____