SUBCLINICAL ATHEROSCLEROSIS IN DIABETIC PATIENTS IN KNH DIABETIC OUTPATIENT CLINIC

Dr Husein M. Tayabali

H58/83349/2015

A Research Proposal Submitted in Partial Fulfillment for the Award of Master of Medicine in Diagnostic Imaging and Radiation Medicine, University Of Nairobi

University of Nairobi, College of Health Sciences,

Department of Diagnostic Imaging and Radiation Medicine.

LIST OF INVESTIGATORS

PRINCIPAL INVESTIGATOR

Dr Husein M. Tayabali,

Department of Diagnostic Imaging and Radiation Medicine.

University of Nairobi.

SUPERVISORS

1. Dr Timothy Musila Mutala,

Department of Diagnostic Imaging and Radiation Medicine,

University of Nairobi.

2. Dr Angeline A. Aywak,

Department of Diagnostic Imaging and Radiation Medicine,

University of Nairobi.

DECLARATION

I, **Dr Husein M. Tayabali,** declare that the work contained herein is my original idea and has not been presented at any other place in Kenya to the best of my knowledge.

Signature…… Date…….....

Approval by Supervisors

This research proposal has been submitted with my approval as a University supervisor;

1. Dr Timothy Musila Mutala,

Consultant Radiologist and Lecturer,

Department of Diagnostic Imaging and Radiation Medicine,

University of Nairobi.

Signature…… Date…….....

2. Dr. Angeline A. Aywak,

MBChB (Nbi) MMed (Nbi),

Consultant Radiologist and Senior Lecturer,

Department of Diagnostic Imaging and Radiation Medicine,

University of Nairobi.

Signature…… Date…….....

ACKNOWLEDGEMENTS

I thank the Almighty God for this life; and the opportunity to make a difference through medicine.

I appreciate my supervisors Dr. Mutala and Dr. Aywak for their commitment and guidance throughout, from development of the protocol to writing this dissertation.

Finally, to my wife, my mother and my daughter, I owe my gratitude for their unwavering support. You are my pillars.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AGE	Advanced Glycation End-products
AR	Aldose Reductase
BMI	Body Mass Index
CAC	Coronary Artery Calcium Score
CAD	Coronary Artery Disease
CIMT	Carotid Intima Media Thickness
CVD	Cardiovascular Disease
DAG	Diacyl Glycerol
DDIRM	Department of Diagnostic Imaging and Radiation Medicine
DM	Diabetes Mellitus
DOPC	Diabetic Outpatient Clinic
ERB	Ethics Review Board
ICAM	Intracellular Adhesion Molecules
IL	Interleukin
IVUS	Intravascular Ultrasound
KNH	Kenyatta National Hospital
LDL	Low Density Lipoproteins
MDCT	Multi-detector Computed Tomography
MI	Myocardial Infarction
MRI	Magnetic Resonance Imaging
NEFA	Non-esterified Free Fatty Acids
OHA	Oral Hypoglycemic Agents
PAD	Peripheral Arterial Disease
PAI-1	Plasminogen Activator Inhibitor-1

PKC	Protein Kinase C
SDH	Sorbitol Dehydrogenase
TGF-B1	Transforming Growth Factor -B1
TNF	Tumor Necrosis Factor
UoN	University of Nairobi
US	Ultrasound
VCAM	Vascular Cell Adhesion Molecule
WHO	World Health Organization

ABSTRACT

Background

Diabetes mellitus is a condition of increasing public health interest due to its rising prevalence, especially within middle and low income communities.

It is recognized that cardiovascular complications are a leading instigator of diabetic morbidity and mortality. As such, early detection of subclinical markers of cardiovascular disease would be valuable in the prevention and therapeutics of said complications. One of the recognized methods of assessing for subclinical atherosclerosis is measuring carotid artery intima-media thickness by use of B-mode ultrasonography.

Objectives

To determine the prevalence of subclinical carotid atherosclerosis using carotid intima media thickness (CIMT) of >0.8 mm, and the associated clinical and cardiovascular risk factors in diabetic patients at the KNH Diabetic Outpatient Clinic.

Methods

This was a hospital based cross-sectional descriptive study carried out at the Kenyatta National Hospital Diabetic outpatient clinic on diabetic adults. Study participants' medical records were examined to obtain socio-demographic and clinical data. Study subjects underwent a clinical assessment of their weight, height, body mass index (BMI), waist circumference, hip circumference, waist hip ratio and blood pressure. Measurement of the carotid intima media thickness (CIMT) and evaluation of carotid plaque was done using B-mode ultrasound.

Outcome Measures

Between March & May of 2019, 384 diabetic patients were recruited into the study. Mean age of study subjects was 57.0 years (SD 13.2). 62.0% of the subjects were female. 244 persons (63.5%) had hypertension, 72 (18.8%) were smokers, 247 (70.6%) had abnormal waist: hip ratios and 261 persons (68.0%) had evidence of subclinical atherosclerosis. Persons with subclinical atherosclerosis as compared to those without, were older ($p < 0.001$), were more likely to have central obesity based on waist hip ratio ($p = 0.004$), and were more likely to be hypertensive($p < 0.067$).

Conclusion

In a fairly young diabetic population, there was a high preponderance of subclinical atherosclerosis. This was largely driven by a high prevalence of traditional cardiovascular disease risk factors. Aggressive screening and management of these risk factors should be assimilated into the everyday care of these individuals.

1 INTRODUCTION

Diabetes is a chronic, progressive metabolic disorder defined by elevated blood glucose levels. It is no longer considered a disease of predominantly affluent nations and its prevalence is on the rise, particularly in middle and low income nations. As of 2014, 422 million people worldwide were suffering from diabetes, and approximately 1.5 million mortalities were associated with diabetes in the same year.[1]

Type 2 diabetes, being the more prevalent form compared to type 1, is characterized by insulin resistance and followed by eventual insufficiency of insulin if uncontrolled. It was previously considered a disease of adulthood but is currently documented in pediatric age-groups as well.[2]

Early symptomatology is due to deranged blood glucose levels; namely polydipsia, polyuria, polyphagia and unintentional weight loss.[3]

A majority of the later symptoms of the disease arise due to vascular complications related to diabetes. Notable is the fact that diabetic patients have a tenfold risk of cardiovascular disease in their lifetimes as compared to individuals not suffering from diabetes.[4]

DM inclines to elevated rates of coronary, cerebral and peripheral vascular disease as well as vascular dementia, diabetic retinopathy and chronic kidney disease. Vigorous blood sugar control has shown mixed results in the halting of cardiovascular sequelae of DM, with some studies even noting no change in CVD risk in patients with tightly controlled blood sugars.[5]

When correlated with non-diabetics as concerns cardiovascular disease, diabetic patients have a greater overall coronary plaque burden and a higher rate of multi-vessel disease.[6]

Subclinical atherosclerosis can be observed using various imaging techniques. B-mode ultrasound of the common and internal carotid arteries is a cheap and reproducible non-invasive technique used to assess thickness of the vascular intima-media complex[7]. This technique has been validated and is in widespread utilization to detect intima-media thickness and presence of plaque. An increased carotid intima media thickness (CIMT) is a representative indicator for atherosclerosis and a predictor of future cardiovascular events[8]. CIMT measurement thus has useful indications including screening for atherosclerosis, risk stratification for future CVD events and appraisal of the efficacy of drugs such as statins and antihypertensive medications.[9]

Considering the social and financial burden on communities and nations worldwide, prevention of the disease must be made a priority, in the form of education on the risk factors pertaining to the same.

However, in individuals already living with the condition, vigilance must be maintained, in order to avoid the dire complications that are attached to the disease, while also actively seeking

opportunities for intervention in order to reduce the damage caused by complications that have already occurred.

2 LITERATURE REVIEW

2.1 Burden of Diabetes

Diabetes mellitus is of growing clinical and public health concern due to its pandemic nature. In 2015 it was estimated that 422 million people were living with diabetes with a global expenditure on the disease and its complications estimated at $1.3 trillion.[10]

In Kenya the prevalence has been estimated at 2.7% in rural areas and up to 10.7% in urban areas, accounting for 27% of all hospital admissions in 2003.[11]

2.2 Diabetes and Cardiovascular Disease (CVD)

2.2.1 Increased Risk of CVD in Diabetes

CVD is one of the dominant causes of mortality in the general population and it is well established that there is a direct correlation between diabetes and cardiovascular disease. The Framingham study concluded that diabetic males have a twofold risk; and diabetic females almost threefold; of developing CVD as compared to non-diabetic individuals.[12]

Diabetes manifests as CVD in a number of ways, including myocardial infarction, coronary artery disease, stroke and transient ischemic attack, diabetic nephropathy, peripheral neuropathy and diabetic foot, and erectile dysfunction.

2.2.2 Pathogenesis of Cardiovascular Disease in Diabetes

There are considerable alterations caused by diabetes on the vasculature as diabetes affects an array of functions at the cellular level including the endothelium and smooth muscle cells, platelets, lipoproteins, clotting factors, triglycerides, as well as local arterial response to hypoxia and new collateral vessel formation.[13]

Apart from chronic hyperglycemia, the pathogenesis of diabetic atherosclerosis also concerns the indirect consequences of insulin resistance, dyslipidemia, hypercoagulability, and defective response to injury. It is this widespread dysfunction that makes the side effects so deleterious and the treatment so difficult.

2.2.2.1 Proatherogenic Mechanisms Associated with Hyperglycemia

The polyol pathway consists of the enzymes aldose reductase (AR) and sorbitol dehydrogenase (SDH). AR reduces toxic aldehydes to inactive alcohols, thereby conserving cells.[14] Under hyperglycemic conditions, AR promotes catalysis of glucose which results in production of sorbitol which is then further metabolized to fructose. This leads to increased osmotic and oxidative stress within the cell and may also promote increased Advanced Glycation End-products (AGE) formation.

A study by Vikramadithyan *et al* demonstrated that the glucose influx through the polyol pathway has been linked to atherosclerosis in LDL receptor (LDLR) deficient mice.[15]

Advanced glycation end products (AGE) are produced by reaction of intracellular dicarbonyls with amino acids. Their formation leads to alteration of proteins within cells relevant to atherogenesis which express receptors for AGE (RAGE).[16 17]

There is a notable increase in transcription of intracellular adhesion molecules such as Intracellular adhesion molecule 1(ICAM-1), Interleukins 1 and 6 and Tumor necrosis factor alpha (TNF alpha) which are known to be pro-atherogenic receptors.[16]

Protein kinase C (PKC) is activated by intracellular hyperglycemia, leading to induction of Diacyl Glycerol (DAG). It can also be incidentally activated by RAGE engagement or polyol pathway activation.

This leads to increased monocyte adhesion to the endothelium, as well as increased expression of thrombin induced ICAM-1.

The hexosamine pathway provides substrates for synthesis of proteoglycans and glycoproteins. Hyperglycemia-induced activation of this pathway has been associated with transcription of transforming growth factor-β1 (TGF-β1) and plasminogen activator inhibitor-1 (PAI-1) in endothelial cells.[18]

12/15-lipoxygenase (12/15-LO) pathway encourages oxidation of LDL rendering it even further atherogenic. It also reduces IL-12 secretion suggesting that it may have a role in inflammatory response, together with promotion of vascular smooth muscle cell hypertrophy.

2.2.2.2 Proatherogenic Mechanisms in Addition to Hyperglycemia

Type 2 diabetes is linked to hyperlipidemia which, as discussed above, leads to increased formation of glycoxidation and AGE products, but despite LDL levels being normal, the composition is modified and thus more atherogenic than in normal individuals. Statin therapy has been noted to decrease cardiovascular events by normalizing lipid profile and other anti-inflammatory effects.

Adiponectin is a cytokine produced by adipose tissue whose serum levels have been noted to be inversely correlated with insulin resistance[19]and may have pro-atherogenic effects.

C Peptide is a decomposition product of proinsulin which is expressed in atherosclerotic lesions in diabetic individuals.[20] It displays chemotactic activity towards monocytes and induces proliferation of vascular smooth muscle cells.

Lipoprotein lipase hydrolyses triglycerides and corresponds inversely with HbA1c. It is considered a good marker integrating diabetes and atherosclerosis.

A majority of the above mentioned mechanisms are currently being used in research for therapeutic agents to curtail the effects of diabetes on the cardiovascular system.

Genetic screens have also identified polymorphisms shared between diabetes and atherosclerosis.[21]

2.3 Subclinical Atherosclerosis

Atherosclerosis is a progressive, inflammatory process causing systemic vaso-occlusive disease. It is characterized by the presence of plaques and occlusive lesions within the intimal layer of vasculature.

It may manifest as ischemic heart disease, unstable angina pectoris, stroke and limb ischemia or gangrene, depending on the vessel affected, whether coronary, cerebral or peripheral.

Atherosclerosis is often first identified in patients who experience a major cardiovascular event.

Subclinical atherosclerosis is a disease entity where the condition is not advanced enough to present with clinically observable symptoms. However, numerous techniques exist which if used can demonstrate subclinical atherosclerosis in its preclinical stages.

A majority of plaques are clinically silent, but if rupture of the plaque occurs, luminal narrowing is found to be more pronounced due to the healing process of the plaque.[22]

Based on the Framingham risk assessment, age is used as a proxy for coronary plaque burden, which is to say that the prevalence of atherosclerosis increases with age, but it is important to note that plaque burden can vary from person to person; and as such exact analysis of subclinical atherosclerosis may improve the prediction of risk for acute cardiovascular events.[23]

It has been noted that half of all men and approximately two thirds of women in America who die suddenly from heart disease were previously healthy and were not categorized as high risk as per the Framingham risk scoring.

While Intima media thickness is highly variable based on age, gender and ethnicity[24], a number of studies have demonstrated the utility of applying 0.8mm as the cutoff for subclinical atherosclerosis. Kota et al found a significant increase in prevalence of occurrence of ischemic stroke in diabetics with CIMT higher than 0.8mm[25].

Five thousand adults aged 65 years and above who participated in the Cardiovascular Health Study were assessed for subclinical atherosclerosis and the reported prevalence of subclinical

atherosclerosis from this study was 36% in women and 38.7% in men with prevalence being noted to increase with age.[26]

A separate study by Jaffer *et al* randomly selected 318 asymptomatic subjects from the Framingham Offspring Study cohort and used cardiovascular magnetic resonance imaging (MRI) to evaluate subclinical aortic atherosclerosis and reported a 38% and 41% prevalence in women and men respectively with prevalence once again increasing with age.[27]

With these points in consideration, a method of measuring subclinical atherosclerosis being integrated into the risk scoring systems currently in use may provide further value in predicting risk for acute cardiovascular events.

2.4 Diagnosis of Subclinical Atherosclerosis

A number of techniques have been described which can measure atherosclerosis, whether subclinical or overt. These techniques vary in that they may be invasive or non-invasive and measure various parameters including luminal diameter, vessel wall thickness and plaque volume.[28]

Table 1: Characteristics of techniques used for identification of subclinical atherosclerosis

Characteristic	B-Mode Ultrasound	Intravascular Ultrasound	Coronary angiography	MRI	Electron Beam CT
Invasive	No	Yes	Yes	No	No
Primary measure	Intima Media Thickness (IMT)	Plaque Volume and Composition	Stenosis	Plaque Volume and Composition	Coronary Artery Calcification (CAC)
Plaque Composition	No	Yes	No	Yes	No
Plaque Burden	No	Yes	No	Yes	Yes
Plaque Vulnerability	No	Yes	No	Yes	No

2.4.1 Techniques for Measuring Subclinical Atherosclerosis

2.4.1.1 Non-Invasive Techniques

B-Mode ultrasonography is generally the most widely accepted method used to assess the common carotid artery, whose intima medial thickness (IMT) reflects diffuse thickening in atherosclerosis and has been corroborated as a risk of disease burden and subsequent cardiovascular events as a result thereof.[29] Serial IMT measurements can also provide data on the efficacy of therapy such as anti-hypertensives and lipid lowering agents.[30]

High resolution MRI is a non-invasive technique that can evaluate atherosclerotic plaque burden by determining the plaque volume, composition and integrity of fibrous cap. Consequently, it also provides a measure of susceptibility to rupture.[31]

ECG-gated multi-detector CT (MDCT) can be used to evaluate the Coronary Artery Calcium score (CAC)[32] which reflects coronary plaque burden, which in turn is an independent cardiovascular risk factor.[33] Unlike MR, CT can only quantify plaque burden but is unable to predict the susceptibility of the plaque to rupture.

2.4.1.2 Invasive Techniques

Coronary angiography is useful in localizing atheromatous plaques and in estimating the degree of coronary luminal stenosis, but it also cannot readily identify plaques susceptible to rupture.[34]

Intravascular ultrasound (IVUS) is where miniature ultrasound transducers are attached to the end of a coronary catheter. This method can be used to assess the size and composition of plaques along the entire thickness of the vessel wall and thus provides information about lesion location and the degree of plaque burden.[35]

2.5 Detection of Subclinical Atherosclerosis Using Carotid Intima Media Thickness

CIMT is thought to be a thorough picture of all changes caused by different risk factors over an interval on the arterial walls[36]. It can therefore be described as a robust indicator of vascular risk.

It is a simple, reproducible and economical tool to determine the incremental effect of atherosclerotic risk factors. It is also an independent predictor of future cardiovascular risk and can identify vulnerable patients who would benefit from aggressive interventions to prevent CVD events[37]

CIMT is a measure of the thickness of the intima-media complex of the carotid artery to detect early atherosclerosis. High frequency linear array transducers (5-15MHz) in B-Mode can be used to visualize the carotids. Longitudinal images are obtained from the near and far walls of the right and left distal common carotid arteries. Using standardized ultrasound equipment, protocols

and dedicated software for image analysis, the inter-scan and inter-observer reproducibility of CIMT measurement is excellent.[38]

2.6 Increased CVD Risk and CIMT

Atherosclerosis is a precursor to vascular complications including; but not limited to, MI, stroke, renovascular and peripheral vascular disease. As such, timely identification of atherosclerotic change may aid in identification of at-risk individuals and subsequently slow the progression of the disease in order to prevent complications.

Studies performed in the general population have shown a gradual increase in CVD risk with increasing CIMT, as modest correlation between increased CIMT and coronary atherosclerosis has been demonstrated(8). In individuals who were not exposed to the traditional risk factors associated with atherosclerosis, these changes were found to be age related. (8, 35) when risk factors were present, an increased relative risk for MI and stroke was noted.

A prospective study by Polak et al opined that the net increase in risk of CVD events after inclusion of the CIMT to the Framingham risk scores was 5.8% and thus concluded CIMT to be an independent predictor of cardiovascular events.[39]

Similarly, a meta-analysis of 8 studies conducted by Lorenz et al, encompassing 37,197 subjects, found the yearly incidence of cardiovascular events to be incremental in individuals in whom subclinical atherosclerosis was detected. (8)

On the other hand, a reduction in CIMT subsequent to initiation of statin therapy has been associated with reduction in cardiovascular events, as has been demonstrated in the REGRESS trials.[40]

2.7 Subclinical Atherosclerosis in DM

The epidemiology of cardiovascular disease when tied in with diabetes is two-tier. Traditional coronary heart disease risk factors such as hypertension, dyslipidemia, and obesity account for part of the risk. However several diabetes specific factors have been shown to increase morbidity and mortality in coronary artery disease.[41] For example, atheromatous plaques in DM are found to be more lipid-rich, with greater macrophage infiltration and thus have greater susceptibility to rupture than in patients without diabetes.[42]

DeLuca et al performed treadmill exercise sestamibi stress tests on known DM patients and detected 40 out of the 217 participants (18%) to have unrecognized MI and silent myocardial ischemia.[43]

Malik et al studied CAC and CIMT in diabetic patients and found their data to corroborate the recommendations of many currently used criteria in assessing CVD risk in asymptomatic adults. Increased calcium scores and intima thickness was found to increase CVD risk by 3-4%.[44]

3 STUDY JUSTIFICATION

The rising prevalence of diabetes mellitus and its associated morbidity and mortality were a growing concern both locally and globally. CVD related to DM being a major determinant towards these complications, it is essential towards management of the condition to be able to diagnose atherosclerotic change in its early stages.

Measurement of CIMT using B-Mode ultrasound was a readily available, relatively inexpensive, safe and easy way to identify at-risk individuals and start appropriate therapies to avoid said complications.

No recorded Kenyan studies had been done to measure the atherosclerotic burden in local diabetic patients. This study aimed to fill the knowledge gap and provide information that may change patient therapies in avoidance of end-stage diabetic sequelae.

If the data from this study could be used to change the standard operating procedures in diabetic patient work-up and screening, it could eventually reduce the severity and even the amount of vascular complications faced by these patients in Kenyatta National Hospital, and possibly nationwide by altering patients risk levels and subsequently tailoring therapies as per their specific needs.

The data obtained from the study would also contribute to the scientific knowledge base worldwide.

4 OBJECTIVES

4.1 Broad Objective

To determine the prevalence of subclinical carotid atherosclerosis and the associated clinical and CVD risk factors in diabetic patients at KNH-DOPC.

4.2 Specific Objectives

1. To determine the prevalence of subclinical carotid atherosclerosis in diabetic patients at KNH-DOPC.

2. To compare prevalence of selected traditional CVD risk factors in diabetic patients who have subclinical carotid atherosclerosis and those without subclinical carotid atherosclerosis.

a. Hypertension
b. Obesity
c. Smoking
d. Dyslipidemia

3. To compare the proportion of diabetic patients with subclinical carotid atherosclerosis and their drug regimens.

5 STUDY METHODS

5.1 Study Design

Hospital based cross-sectional study.

5.2 Study Area

a. Kenyatta National Hospital, Diabetic Outpatient Clinic.

The clinic was relocated outside of the main hospital block for purposes of decongestion. It ran on Monday, Tuesday, Thursday and Friday from 8am to 4pm and an average of 400 patients were seen per month.

The patients were attended to by trained nurses, clinical and medical officers as well as registrars training in internal medicine.

The patients also received nutrition and general counseling. Patients' medical records were handled by qualified data clerks and records officers.

b. Department of Diagnostic Imaging and Radiation Medicine, University of Nairobi.

The department was located within the old hospital. There were 3 functioning ultrasound machines.

The department was run primarily by registrars in radiology who were overseen by consultant radiologists.

The patients having been directed to the department would be seen by the principal investigator who performed carotid ultrasounds on a single dedicated ultrasound machine with specific vascular intima measuring software installed which was able to assist in accurate data retrieval and collection.

Once the results were obtained they were retained so as to transfer relevant data into the patients file.

5.3 Sample Size

For large populations (i.e. 10,000 and above), sample size for descriptive study was estimated as:

$$n_0 = \frac{Z^2 * p(1-p)}{e^2}$$

[Cochran (1963)]

Where,

n_0 -sample size for target population >10,000;

Z - Standard variate (1.96) which corresponds to 95% confidence interval;

e - The desired level of precision; and

p - Estimated prevalence of subclinical carotid atherosclerosis in diabetic patients at KNH-DOPC.

This study desired a 95% confidence level (z=1.96) and ±5% precision. We assumed p=0.5 since there was no similar study conducted in Kenya.

Substituting the above parameters, the sample size became:

$$n_0 = \frac{1.96^2 * 0.5(1-0.5)}{0.05^2} = 385$$

384 participants were recruited.

5.4 Sampling Procedure

Convenience sampling was used with the researcher stationed in the clinic enrolling all diabetic patients meeting eligibility criteria into the study consecutively until the desired sample size was achieved.

5.5 Inclusion Criteria

- All type 2 diabetic patients at KNH DOPC aged >18years.

5.6 Exclusion Criteria

- Patients with known atherosclerotic vascular disease i.e. those with previous stroke, MI or peripheral artery disease.
- Patients who declined to consent.

5.7 Methodology

A cross sectional descriptive study was conducted on patients visiting the diabetic outpatient clinic in KNH who met the specified criteria.

Patient selection was done by convenience sampling at the diabetic outpatient clinic where a detailed history was extracted from eligible individuals and relevant information concerning the procedure was discussed.

The sampling was done from Monday to Friday, when the Diabetic clinic functions and all information was extracted before the patient had their doctor's visit. They were then advised by the principal investigator or research assistant to come to the Radiology Department for their carotid ultrasound on completion of their clinic visit, preferably the same day in the afternoon, so as to avoid repeat travel to KNH.

The eligible patients, after having filled the data collection tool (which was used to obtain the patients' demographic data and a concise medical history), were expected to sign a written informed consent form, after which they were directed to the UoN-DDIRM where a clinical evaluation and B-Mode ultrasound was performed to assess for subclinical atherosclerosis in the carotid arteries bilaterally.

Relevant medical history to assess CVD risk i.e. hypertension, dyslipidemia, lifestyle characteristics (e.g. cigarette smoking and alcohol use) and family history of CVD were obtained. The most recent lipid profiles and HbA1c testing done on the study subjects were recorded.

The patients' medical records were also studied to identify pertinent information such as duration of illness, co-morbidities, type of medication used for glycemic control and blood pressure control; where applicable; as well as other medication used such as lipid lowering agents.

5.8 CIMT Measurement

Patients that were included into the study were invited to the University of Nairobi's Department of Radiology where B-Mode ultrasound of the carotid arteries was done bilaterally using standard protocol. The examination was conducted by the principal investigator using a linear array transducer (6-13MHz) on a GE Logiq S7 ultrasound machine.

The examination was conducted by the principal investigator at no cost to the patient as had been discussed with the DDIRM; of which the principal investigator was a resident.

5.9 Technique

The examination was explained to the patient after which they were asked to lie on the couch supine with their neck extended. Bilateral examination of the carotid vessels was done for each patient. The neck was rotated to the opposite side of the examination and initial transverse scan was done in B-Mode to identify the vasculature after which a longitudinal scan was used to identify the common carotid artery, the carotid bulb and bifurcation. The vessels were examined for overt plaque formation or stenosis and intimal thickness was measured 10mm proximal to the carotid bulb. Measurements were taken from both the near and far walls in two longitudinal views; parallel to the anterior and posterior borders of the sternocleidomastoid muscle respectively.[45]

CIMT was measured using electronic calipers assisted by specialized proprietary software from General Electric on the aforementioned Logiq S7 machine used to identify a long segment of intima and take a maximal and mean measurement of every segment measured.

A brief training session was held with the consultant radiologists to confirm adequacy of scanning technique and proficiency in use of the software for measurement of CIMT.

An intimal measurement of greater than 0.8mm was considered abnormal and a measurement of greater than 1.5mm was reported as plaque formation. (24)

The results obtained from the ultrasound, whether normal or abnormal, were rendered into the relevant patients' file for appropriate action by the clinician.

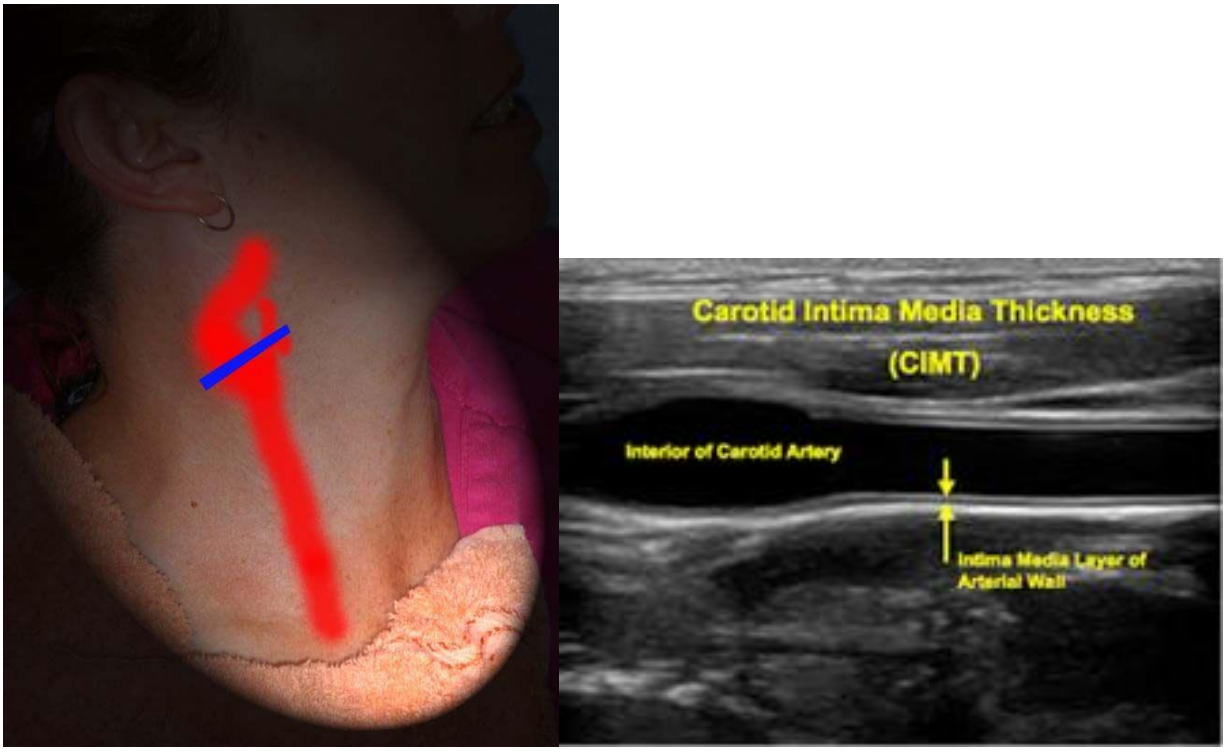


Figure 1a and 1b: This illustrates the scan plane used to attain the image of the carotid vessels and the expected image of the carotid artery with a well-defined intimal layer.

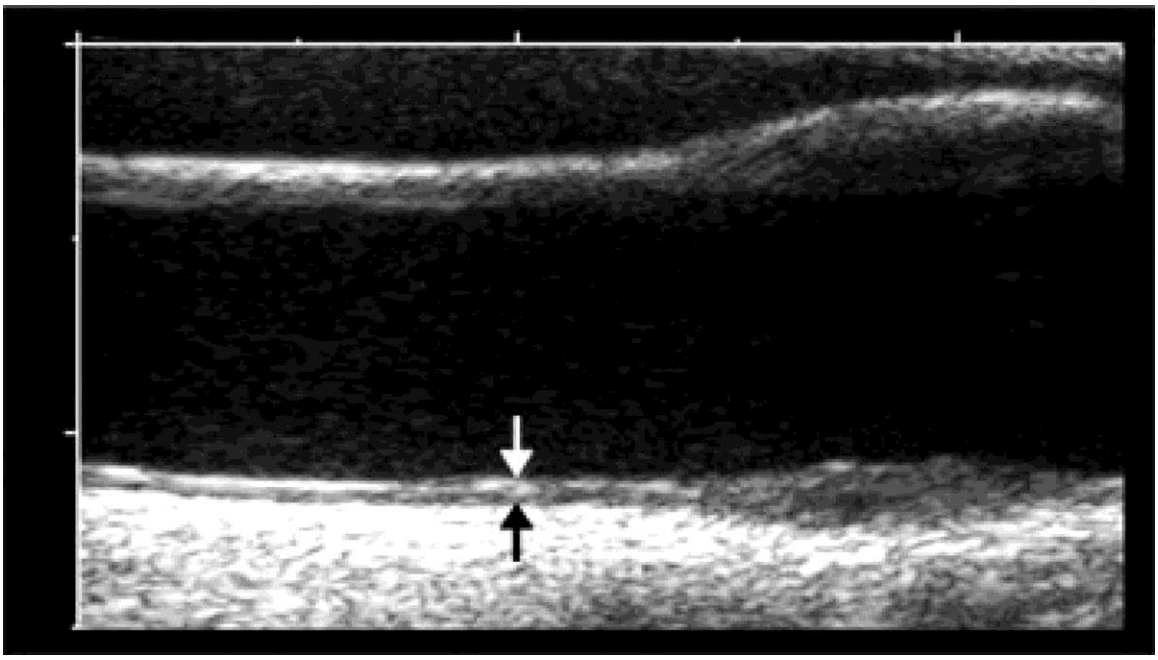


Figure 2: A zoomed-in image to illustrate the intima of the common carotid artery.

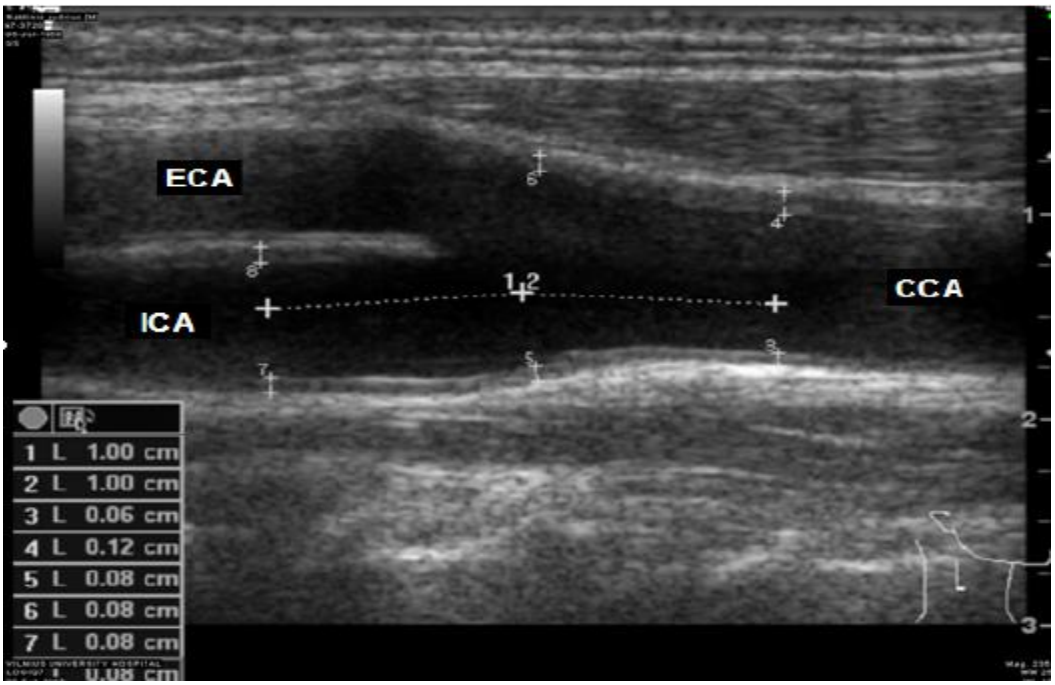


Figure 3: Automated software taking numerous measurements over a desired segment of the vessel to give a maximum, minimum and mean of the intimal thickness.

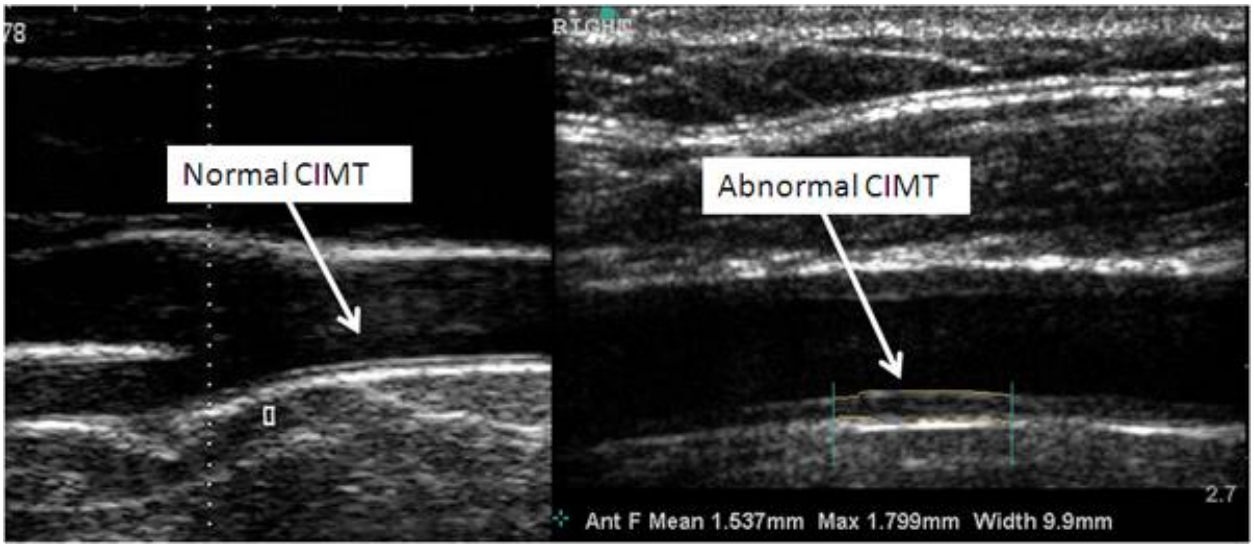


Figure 4: The thickness of the intimal layer is greater than 8mm and as such is labeled abnormal.

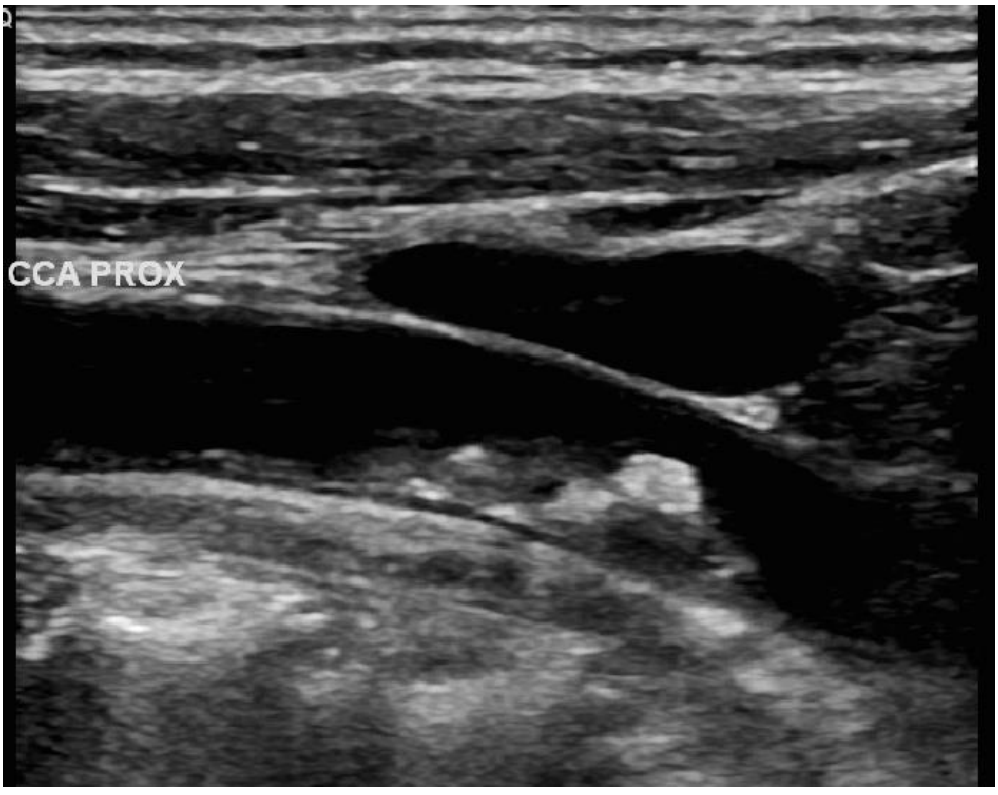


Figure 5: There is a focal large irregular thickening of the intima media of the vessel under scrutiny. This will be labeled as a carotid plaque.

5.10 Anthropometric Measurements

Apart from the above mentioned clinical history and imaging, pertinent measurements were done in a standardized manner as described below:

Height

Standing height was measured once to the nearest 0.5cm barefoot with the back square against the wall tape and a set square resting on the scalp and against the wall.[46]

Weight

Weight was measured once to the nearest 100 grams using a lever balance, barefoot, in light garments. (45)

Body Mass Index

The body mass index (BMI) was calculated using the World Health Organization (WHO) criteria as weight (in kilograms) divided by height (in meters) squared. (45)

Waist Circumference

The waist circumference was measured using a standard tape measure half way between the lowest rib and the iliac crest. (45)

Hip Circumference

Hip circumference was measured as the broadest circumference below the waist using a standard tape measure. (45)

Waist Hip Ratio

Waist hip ratio is the proportion of the circumference of the waist to that of the hips. This was calculated as waist circumference (in centimeters) divided by the hip circumference (in centimeters).

5.11 Definition of Study Variables

Dependent Study Variables

- Subclinical atherosclerosis was defined as CIMT greater than 0.8 mm.
- Carotid plaque was defined as CIMT greater than 1.5 mm.[47]

Independent Study Variables

Age

Age as a CVD risk factor, was defined as:

- Males older than 45 years
- Females older than 55 years

Individuals who were greater than the above mentioned ages were considered to be at an added risk for cardiovascular disease.

Obesity
Overweight and obesity were defined as a BMI 25-29.9kg/m2 and >30 kg/m2 respectively.

Hypertension
Participants in the study were considered hypertensive if they had a Systolic BP of 140 mmHg and above or a Diastolic BP of 90mmHg and above, or if they were on treatment with antihypertensive medication.

Dyslipidemia
The NCEP/ATP III guidelines were used to classify study subjects according to lipid profile status[48]

5.12 Data Analysis

5.12.1 Data collection, entry and storage

After obtaining ethical approval and permission from Kenyatta National Hospital management, data was obtained from study participants after consenting by the research assistant using a structured questionnaire. Participant's identifiers e.g. names, patients' hospital number were not documented in the questionnaires for the sake of confidentiality.

Questionnaires were analyzed daily by the principal investigator to ensure they were completed appropriately. Data collected was posted into an Excel spreadsheet in a key protected computer. Back-up copies were stored in a flash drive which was in the sole care of the principal investigator.

5.12.2 Data Analysis Plan

Data was entered, managed and analyzed in SPSS version 21.0 software. Descriptive characteristics of the study population were presented using summaries of socio-demographic and clinical variables. Categorical variables were summarized into proportions and continuous data was summarized into means or medians. Prevalence of subclinical atherosclerosis was analyzed as a percentage with 95% confidence. Patients with subclinical atherosclerosis (CIMT >0.8 mm) were described using age, gender and duration of exposure to diabetic medication, and then compared with patients with normal CIMT. Furthermore, prevalence of selected CVD risk

factors including hypertension, obesity and smoking were determined. Categorical data was compared between groups using Chi square test or Fisher's exact test in case of small numbers. Means were compared using Student's t test and medians using Mann Whitney U test. Multivariate analysis using logistic regression model was done to determine the factors independently associated with an increased CIMT while controlling for confounders. All statistical tests were performed at 5% level of significance.

6 ETHICAL CONSIDERATIONS

The study was commenced once authorization was received from the Kenyatta National Hospital/University of Nairobi Ethics and Research Review Committee as well as from KNH administration.

Study participants were recruited into the study voluntarily. As such a consent form was issued to those willing to participate, that informed them of their rights as concerns the study, which they were required to read and sign.

Patient confidentiality was maintained during the course of the study. The patients' private information was withheld and was not used for any purpose other than to fulfill the objectives of the research. Patient questionnaires were marked with a unique patient identification number for purposes of data analysis.

7 RESULTS

7.1 Characteristics of study patients

Between March and May of 2019 a total of 463 patients were screened for eligibility at the diabetic clinic. 36 patients were excluded due to previous history of a cardiovascular event and 43 patients declined to consent, leaving a final number of 384 patients who were enrolled for carotid ultrasonography and final analysis.

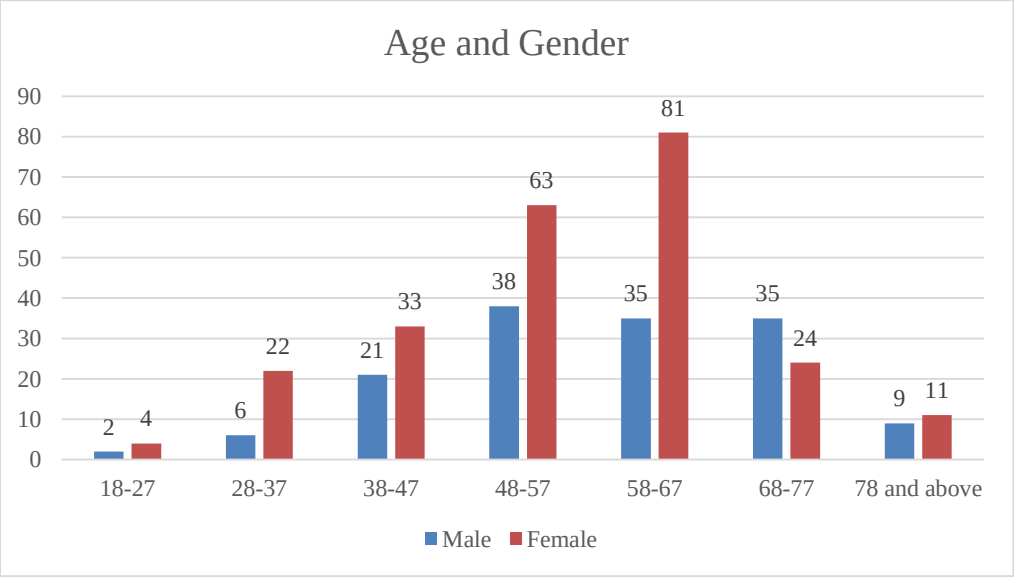
7.2 Sociodemographic Characteristics

The mean age of the patients was 57.0 (SD=13.2) years, while the median age was 58 (IQR=17) years with a range of 18-96 years. 238 participants (62.0%) were female. Approximately two thirds of our participants (63.5%) had at least a secondary education. Only about half of the participants (58%) had some source of income, being either self-employed or employed.

Table 2: Sociodemographic characteristics

Summary of characteristics	Frequency	Percent
Age		
18-27	28	7.3
28-37	54	14.1
38-47	101	26.3
48-57	116	30.2
58-67	59	15.4
68-77	20	5.2
78 and above	28	7.3
Gender		
Male	146	38.0
Female	238	62.0
Marital status		
Single	112	29.2
Married	272	70.8
Occupation		
Employed	221	57.6
Unemployed	163	42.4
Education		
None	26	6.8
Primary	114	29.7
Secondary	192	50.0
Tertiary	52	13.5

Figure 6: Graphical analysis of age and gender distribution of the study population



7.3 Prevalence of Subclinical Carotid Atherosclerosis

All study participants had carotid ultrasound imaging performed. 261 patients (68.0%) had evidence of subclinical carotid atherosclerosis and carotid plaque was observed in 173 (45.1%) patients.

The mean age of patients with subclinical carotid atherosclerosis was significantly higher than patients without subclinical atherosclerosis (normal= 53.4 years; abnormal= 58.7 years; $p < 0.001$).

Table 3: Prevalence of Subclinical Carotid Atherosclerosis

	Frequency	**Percent**
Normal	59	15.4
Single	64	16.7
Multiple	261	68.0
Total	**384**	**100.0**

Individuals who were found to have only a single measurement above the cutoff of 0.8mm were deemed normal, as this single figure out of a total sixteen measurements was taken as either incidental, or erroneous.

Table 4: Prevalence of Plaque Formation

	Frequency	Percent
No plaque	211	54.9
Plaque (focal measurement >1.5mm)	173	45.1
Total	**384**	**100.0**

7.4 Risk Factors and Subclinical Carotid Atherosclerosis

Majority of the patients had more than 2 CVD risk factors. In patients with subclinical carotid atherosclerosis, the commonest CVD risk factor was obesity (by waist hip ratio) which was observed in 75.3%, followed by age 72%, female gender 60%, hypertension 49.6%, smoking 18.4%

Hypertension was present in 244 (63.5%) study participants. It was a significant factor associated with subclinical carotid atherosclerosis in our population. The mean systolic blood pressure in patients with an abnormal CIMT was considerably higher than those with a normal CIMT ($p = 0.067$). While the findings in raised diastolic blood pressure were not deemed significant ($p=0.303$)

72 (18.8%) patients in the study population were classified as current or former smokers. 48 of these had an abnormal CIMT. In this data set, smoking status was not associated with an increased risk of subclinical carotid atherosclerosis (OR 1.1; 95% CI 0.6-1.9; $p = 0.793$).

18.5% of the study population had abnormal waist hip ratios as described by the WHO. In our models, patients with subclinical atherosclerosis were highly likely to be termed obese based on abnormal W/H ratios (OR 2.0; 95% CI 1.2-3.2; $p = 0.004$).

Of the 100 patients who had valid HbA1C measurements, 64 were above the cutoff for appropriate glucose control. Of these 64, 46 (67%) had abnormal CIMT measurements.

Dyslipidemia was not analyzed as part of the data due to the fact that only 41 of the 384 participants had lipid profiles done in the 3 months prior to this study.

On univariate analysis, the odds ratios for waist hip ratio were more than 2-fold. However statistical significance was attained in hypertension, waist hip ratio, alcohol consumption and age. Risk factors that were significant at univariate analysis (age, hypertension, alcohol and W/H ratio) were entered into a multivariate analysis model to determine the factors independently

associated with subclinical atherosclerosis. Only age and W/H ratio were independently associated with subclinical atherosclerosis on multivariate analysis using logistic regression.

Table 5: Comparison of Risk Factors and Subclinical Carotid Atherosclerosis

	CIMT		Total	OR (95% CI)	p-value
	Normal	Abnormal			
Systolic					
≤140	92 (77.3)	175 (68.1)	267 (71.0)	1.6 (0.9-2.6)	0.067
>140	27 (22.7)	82 (31.9)	109 (29.0)		
Diastolic					
≤90	104 (87.4)	214 (83.3)	318 (84.6)	1.4 (0.7-2.6)	0.303
>90	15 (12.6)	43 (16.7)	58 (15.4)		
BMI					
Underweight (<18.5)	2 (1.6)	2 (0.8)	4 (1.0)	2.1 (0.3-15.3)	0.597
Normal (18.5-24.9)	40 (32.5)	63 (24.3)	103 (27.0)	1.5 (0.9-2.4)	0.092
Overweight (25.0-29.9)	29 (23.6)	105 (40.5)	134 (35.1)	0.5 (0.3-0.7)	0.001
Obese (>=30.0)	52 (42.3)	89 (34.4)	141 (36.9)	1.4 (0.9-2.2)	0.134
Waist hip ratio					
Normal	44 (39.6)	59 (24.7)	103 (29.4)	2.0 (1.2-3.2)	0.004
Abnormal	67 (60.4)	180 (75.3)	247 (70.6)		
Smoking					
Yes	24 (19.5)	48 (18.4)	72 (18.8)	1.1 (0.6-1.9)	0.793
No	99 (80.5)	213 (81.6)	312 (81.3)		
Alcohol					
Yes	24 (19.5)	72 (27.6)	96 (25)	0.6 (0.4-1.1)	0.088
No	99 (80.5)	189 (72.4)	288 (75)		
Gender					
Male	42 (34.1)	104 (39.8)	146 (38.0)	0.8 (0.5-1.2)	0.283
Female	81 (65.9)	157 (60.2)	238 (62.0)		
Age and Gender					
Risk (M>45, F>55)	65 (52.8)	188 (72.0)	253 (65.9)	0.4 (0.3-0.7)	<0.001
No Risk	58 (47.2)	73 (28.0)	131 (34.1)		
HBA1c					
Normal	13 (41.9)	23 (33.3)	36 (36.0)	1.4 (0.6-3.5)	0.407
Abnormal	18 (58.1)	46 (66.7)	64 (64.0)		

Figure 7: Prevalence of subclinical atherosclerosis compared to the age of the study population

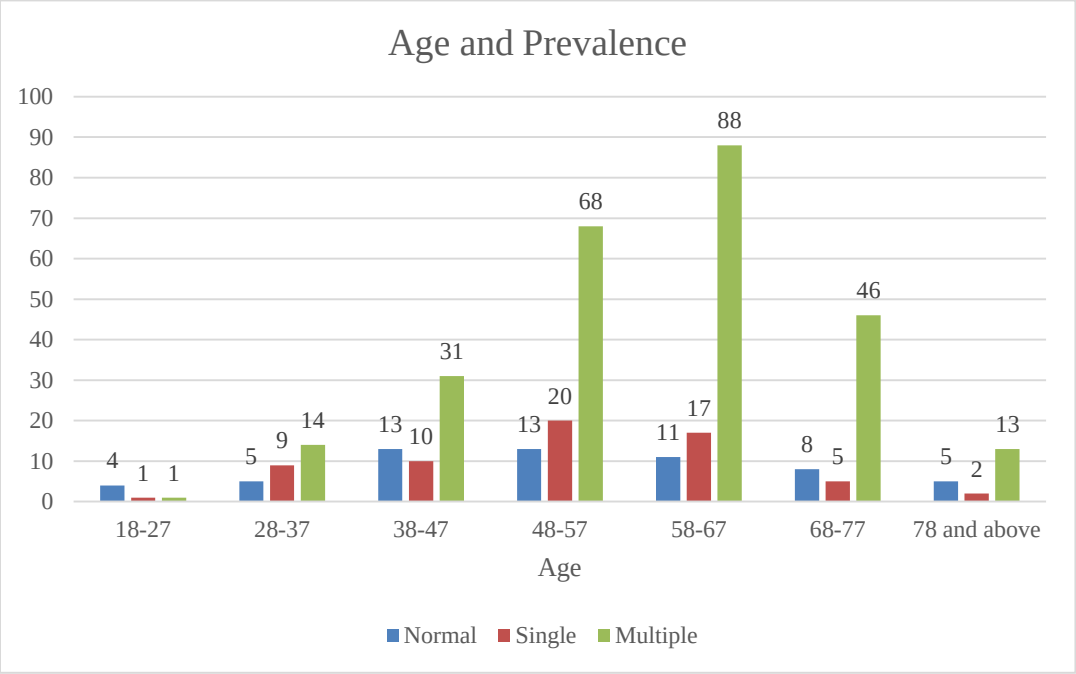


Figure 8: Prevalence of subclinical atherosclerosis compared to systolic blood pressure in the study population

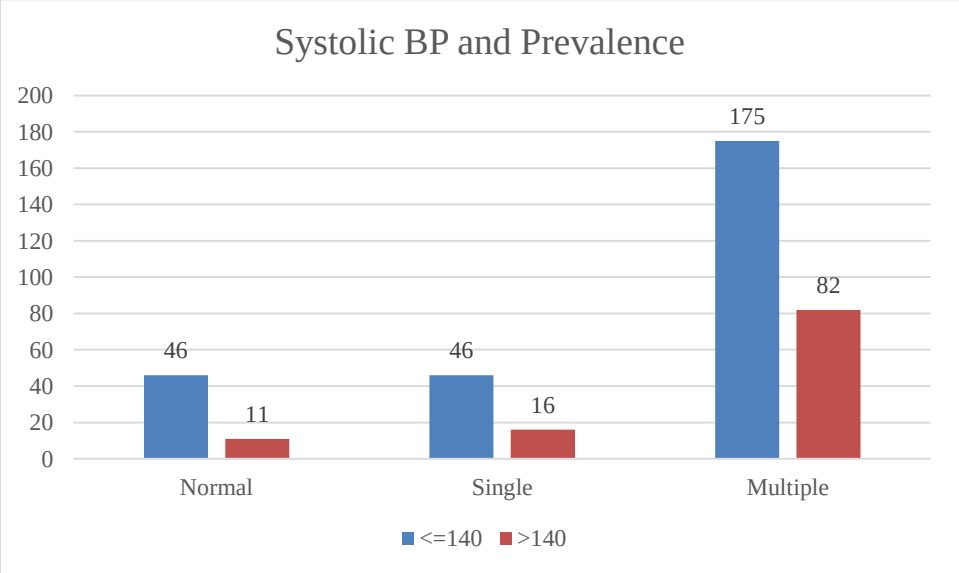


Figure 9: Prevalence of subclinical atherosclerosis compared to diastolic blood pressure in the study population

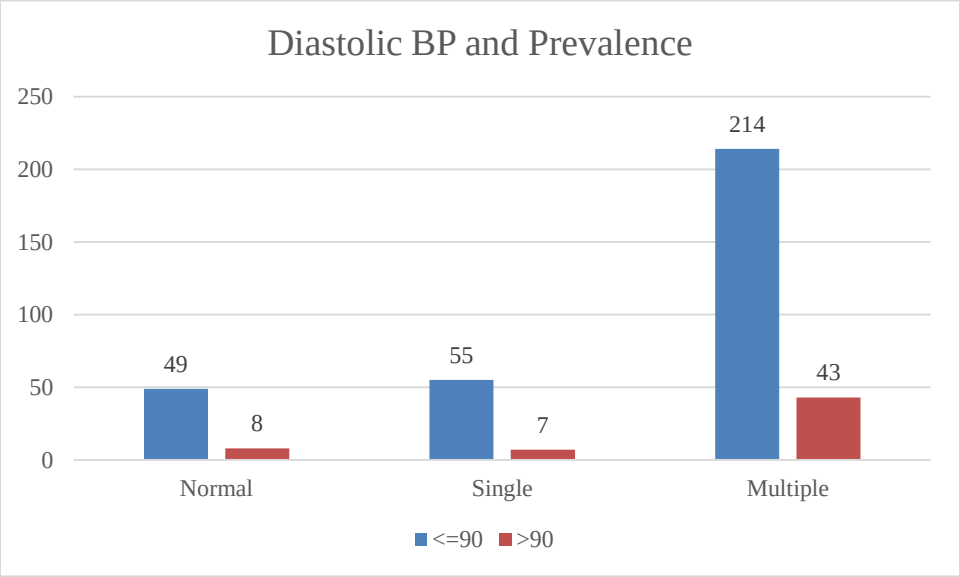
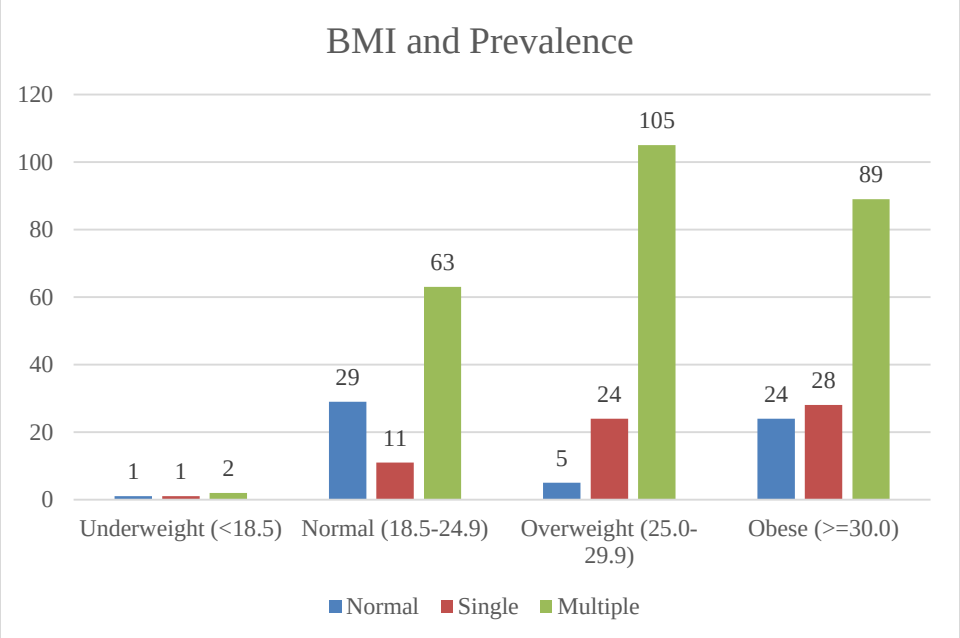


Figure 10: Prevalence of subclinical atherosclerosis compared to body mass index in the study population



7.5 Drug Regimens and Subclinical Atherosclerosis

250 (65%) patients were on antihypertensive drugs; 156 (40%) on statins and 72 (18%) on anti-platelet drugs. In our models, patients on one or a combination of these drugs; and those not on said drugs; were equally likely to have subclinical atherosclerosis.

Table 6: Comparison of Drug Regimens and Subclinical Atherosclerosis

The results of the drug regimens and subclinical carotid atherosclerosis are as shown by the table below.

	CIMT		Total	OR (95% CI)	p-value
	Normal	Abnormal			
BP Drugs					
Yes	78 (63.4)	172 (65.9)	250 (65.1)	0.9 (0.6-1.4)	0.633
No	45 (36.6)	89 (34.1)	134 (34.9)		
Anti-cholesterol					
Yes	45 (36.6)	111 (42.5)	156 (40.6)	0.8 (0.5-1.2)	0.316
No	78 (63.4)	150 (57.5)	228 (59.4)		
Anti-platelets					
Yes	18 (14.6)	54 (20.7)	72 (18.8)	0.7 (0.4-1.2)	0.156
No	105 (85.4)	207 (79.3)	312 (81.3)		

8 DISCUSSION

This study set out to determine the prevalence of subclinical carotid atherosclerosis in diabetic patients and explore associations with traditional CVD risk factors at a resource limited setting in Kenya. Of the population in the study group, 68.0% prevalence of subclinical carotid atherosclerosis was found.

Our prevalence was comparable to that recorded in a recent study by Hong *et al* in Korea, who found a prevalence of 72% while assessing for subclinical atherosclerosis using CIMT.[49] Similarly advanced age and an elevated waist: hip ratio were found to be significant risk factors for subclinical atherosclerosis.

The prevalence was also comparable to studies that used Coronary Artery Calcium (CAC) to evaluate for atherosclerosis. A study by Khaleeli et al on 168 diabetic persons showed a prevalence of 72% of coronary calcium; a marker of atherosclerosis[50]. Over 80% of patients in their study were smokers, and had poor control of dyslipidemia, both of which remain important CVD risk factors.

We explored CVD risk factors in the patients with subclinical atherosclerosis and found an increased prevalence of traditional cardiovascular risk factors. Patients with subclinical atherosclerosis, as compared to those without, were older (Males >45 years and females > 55 years; $p < 0.001$), were more likely to be overweight, based on waist: hip ratio ($p = 0.004$) and were more likely to be concurrently hypertensive.

In a cross-sectional study Perez *et al* found discordance in CVD risk as shown by CIMT as compared to the ASCVD risk score in a diabetic population, with a greater number of patients being classified as high risk using the CIMT.[51] This suggests that using traditional risk scoring systems could possibly underestimate the actual CVD risk in diabetic patients. A possible explanation to these observations is the fact that the aforementioned risk prediction tools only take into consideration traditional CVD risk factors whereas the diabetic population could have combination of other risk factors related to inflammation and metabolic disturbances that accentuate the CVD risk.

Our study population was fairly young (mean age 57 years) and comprised predominantly of females (62.0%) although gender was not found to be of statistical significance to the occurrence of subclinical atherosclerosis in this study.

In this study, traditional CVD risk factors played a key role in their association with subclinical carotid atherosclerosis. Patients with subclinical atherosclerosis were older. The impact of increasing age on subclinical atherosclerosis as measured by CIMT has previously been demonstrated in several studies. Junyent *et al* demonstrated that both the chronological age and vascular age are significantly associated with carotid atherosclerosis in diabetic patients.[52]

Hypertension was a key factor associated with subclinical atherosclerosis in our study. The overall prevalence of hypertension in our patients was 63.5%. A local study in the general population (mean age 33.4 years) conducted in an urban slum in Nairobi, found hypertension prevalence rate of 23%.[53] The higher prevalence in these diabetic patients could be explained by the general increase in traditional risk factors, most prominent of which are increasing age and obesity. An association between hypertension and cardiovascular complications is already apparent at diagnosis of diabetes.

The study showed a strong association between obesity in diabetes (more so when measured by waist: hip ratio than by BMI) and subclinical atherosclerosis. A number of previous studies in diabetic populations have previously demonstrated an increased CIMT with obesity, with more emphasis being placed on central obesity rather than general obesity.[54 55 56]

The prevalence of smokers in our study was 19% and did not show significant association with subclinical atherosclerosis (P=0.793). Vasculopathy associated with smoking has been described in the literature as a strong risk factor for the development and progression of carotid lesions.[57] The lack of association in our study could be due to the fact that most of the patients included as smokers had less than 5 pack years of smoking history. Notwithstanding this low rate of cigarette use in our models, smoking remains a critical cardiovascular hazard that should be tended to in every patient as part of lifestyle modification therapy

The use of cardio-protective medications, namely statins and anti-platelet medication did not reveal any statistical significance. However this could be due to the fact that ours was a cross-sectional study. A study done by Fang et al revealed a regression (but not normalization) in CIMT measurement in individuals who were on atorvastatin for a period of one year.[58]

In conclusion, it is important to note that a combination of history and medical examination to determine traditional CVD risk factors as well as radiological determination of subclinical atherosclerosis are beneficial in appropriate risk assessment and subsequent protocolling of therapies for diabetic patients as demonstrated by the Atherosclerosis Risk in Communities study (ARIC) of 13,145 subjects which opined that the best model to predict incident cardiovascular events included traditional risk factors, CIMT, and plaque[59].

9 CONCLUSIONS

1. A high prevalence of subclinical carotid atherosclerosis in diabetic patients was recorded.

2. Advancing age, hypertension and obesity were demonstrated to have an incremental effect on increasing CIMT and thus were considered significant cardiovascular risk factors in diabetic patients.

10 LIMITATIONS OF STUDY

1. The study design was cross-sectional in nature therefore may not bring out associations with progression of disease.

2. The study was held within the setting of a referral hospital and as such the population studied may be skewed toward a more complex pathology.

11 IMPLICATION OF STUDY & RECOMMENDATIONS

We found a relatively high burden of atherosclerotic cardiovascular disease in our diabetic cohort. This was largely driven by traditional CVD risk factors such as advancing age, hypertension and obesity. Optimizing blood pressure control and treating dyslipidemias are potential targets for intervention. If left untreated these factors can lead to clinical cardiovascular events such a stroke and myocardial infarctions in these patients, thus emphasis needs to be laid on screening and timely management of the aforementioned risk factors, to recognize individuals at an increased risk of future CVD events.

Incorporation of carotid ultrasound into the screening protocols for diabetic patients should be taken into consideration.

Further prospective studies in a larger diabetic population should be carried out to provide incremental information about the risk factors of atherosclerosis, and document morbidity or mortality from cardiovascular disease in this population.

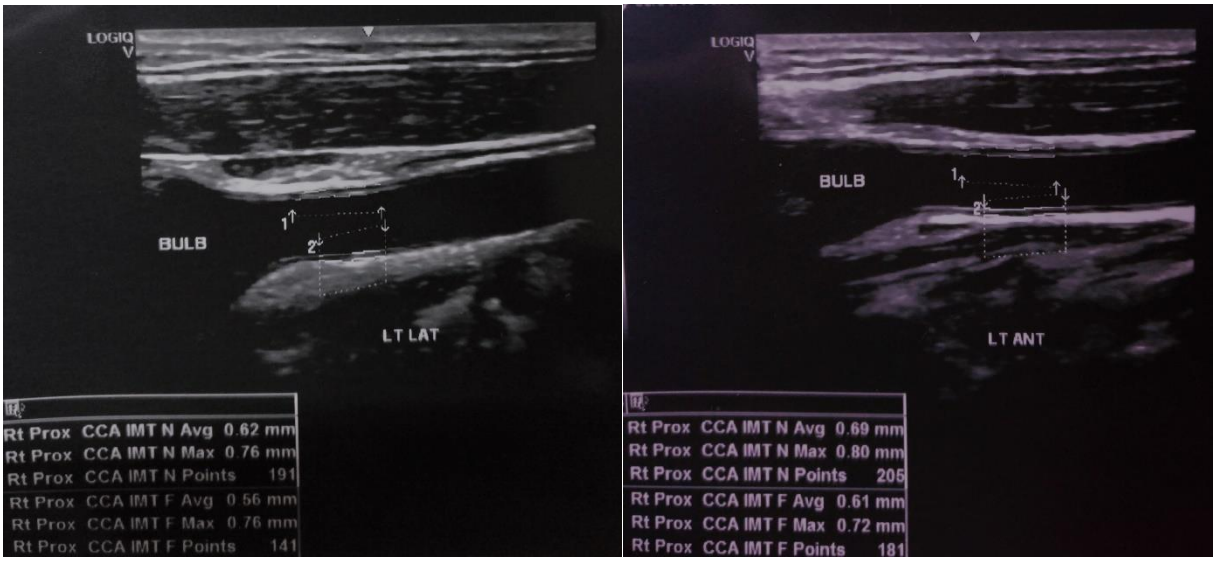


Figure 11a and 11b: Representative images of normal CIMT measurements

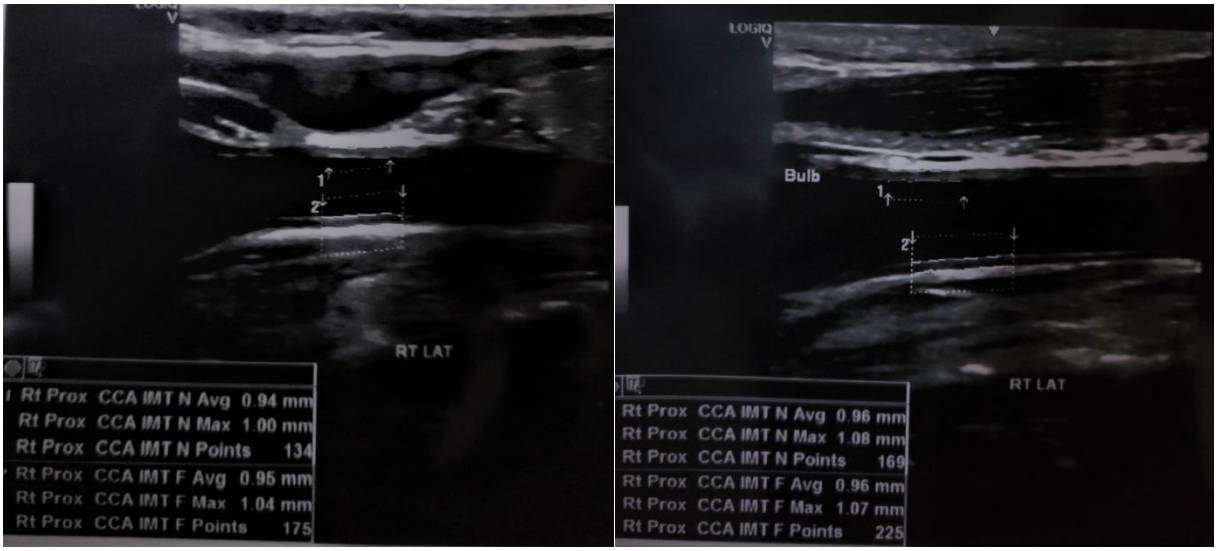


Figure 12a and 12b: Representative images of thickened carotid intima-media complex

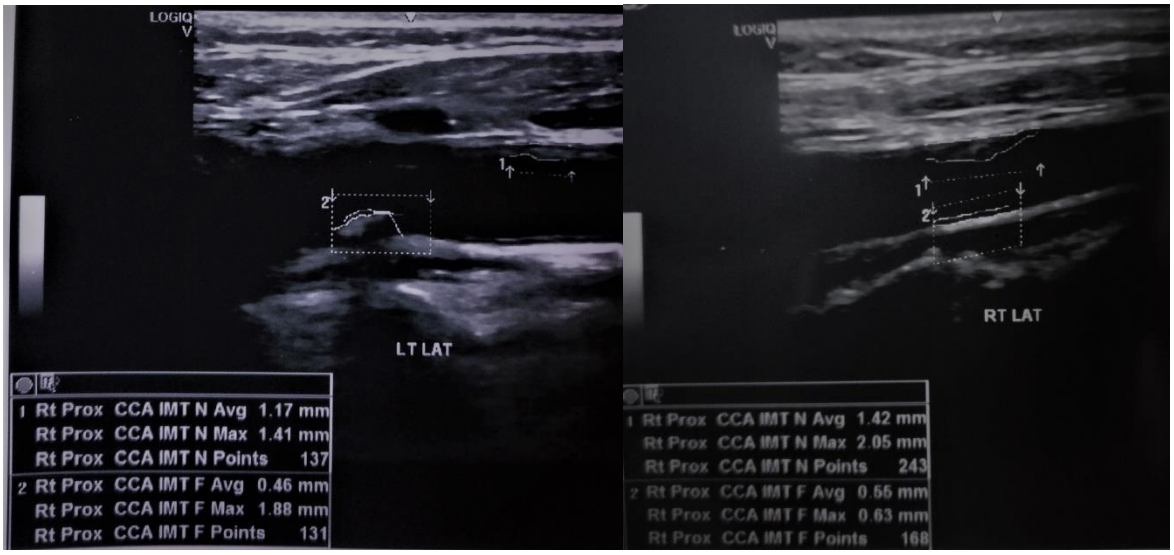


Figure 13a and 13b: Representative images of carotid plaque

REFERENCES

[1] Roglic G. Global report on diabetes. World Heal Organ [Internet]. 2014;58(12):1–88.

[2] Li L-X, Zhao C-C, Ren Y, Tu Y-F, Lu J-X, Wu X, et al. Prevalence and clinical characteristics of carotid atherosclerosis in newly diagnosed patients with ketosis-onset diabetes: a cross-sectional study. Cardiovasc Diabetol [Internet]. 2013 Jan 16 [cited 2019 May 26];12(1):18.

[3] Vijan S. Type 2 Diabetes. Ann Intern Med [Internet]. 2010 Mar 2 [cited 2019 Mar 3]; 152(5):ITC3-1.

[4] Huysman F, Mathieu C. Diabetes and peripheral vascular disease. Acta Chir Belg. 2009;109(5):587–94.

[5] ACCORD Study Group, Long-term effects of intensive glucose lowering on cardiovascular outcomes. N Engl J Med. 2011;364(9):818–28.

[6] Gao Y, Lu B, Sun ML, Hou ZH, Yu FF, Cao HL, et al. Comparison of Atherosclerotic Plaque by Computed Tomography Angiography in Patients With and Without Diabetes Mellitus and With Known or Suspected Coronary Artery Disease. Am J Cardiol [Internet]. 2011 Sep [cited 2019 Mar 4];108(6):809–13.

[7]Heiss G, Sharett AR, Barnes R et al. Carotid atherosclerosis measured by B-mode ultrasound in populations: associations with cardiovascular risk factors in the ARIC study. Am J Epidemiol 1991;134: 250–6.

[8] Sitzer M, Lorenz MW, Rosvall M, Markus HS, Bots ML. Prediction of Clinical Cardiovascular Events With Carotid Intima-Media Thickness. Circulation. 2007;115(4):459–67.

[9] Naqvi TZ, Lee M-S. Carotid Intima-Media Thickness and Plaque in Cardiovascular Risk Assessment. JACC Cardiovasc Imaging [Internet]. 2014 Oct [cited 2019 Mar 4];7(10):1025–38.

[10]Dagogo-Jack S, editor. Diabetes Mellitus in Developing Countries and Underserved Communities [Internet]. Cham: Springer International Publishing; 2017 [cited 2019 Mar 4].

[11] Index @ Www.Nation.Co.Ke [Internet]. 2015.

[12] Gordon T. Premature Mortality From Coronary Heart Disease. JAMA [Internet]. 1971 Mar 8 [cited 2019 Mar 4];215(10):1617.

[13] Beckman JA, Creager MA, Libby P. Diabetes and Atherosclerosis. JAMA [Internet]. 2002 May 15 [cited 2019 Mar 4];287(19):2570.

[14] Spycher SE, Tabataba-Vakili S, O'Donnell VB, Palomba L, Azzi A. Aldose reductase induction: A novel response to oxidative stress of smooth muscle cells (The FASEB Journal (1997) (181-188)). FASEB J. 2012;26(6):2719.

[15] Gleissner CA, Galkina E, Nadler JL, Ley K. Mechanisms by which diabetes increases cardiovascular disease. Vol. 4, Drug Discovery Today: Disease Mechanisms. 2007. p. 131–40.

[16] Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced Glycation End Products. Circulation [Internet]. 2006 Aug 8 [cited 2019 Mar 4];114(6):597–605.

[18] Du X-L, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, et al. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces

plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. Proc Natl Acad Sci [Internet]. 2000 Oct 24 [cited 2019 Mar 4];97(22):12222–6.
[19] Kershaw EE, Flier JS. Adipose Tissue as an Endocrine Organ. J Clin Endocrinol Metab [Internet]. 2004 Jun [cited 2019 Mar 4];89(6):2548–56.
[20] Marx N, Walcher D, Raichle C, Aleksic M, Bach H, Grüb M, et al. C-Peptide Colocalizes with Macrophages in Early Arteriosclerotic Lesions of Diabetic Subjects and Induces Monocyte Chemotaxis In Vitro. Arterioscler Thromb Vasc Biol [Internet]. 2004 Mar [cited 2019 Mar 4]; 24(3):540–5.
[21] Nissen SE, Wolski K. Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. N Engl J Med [Internet]. 2007 Jun 14 [cited 2019 Mar 4]; 356(24):2457–71.

[22] Bhatt S, Tandon A, Bhargava S. Role of imaging in subclinical atherosclerosis. J Int Med Sci Acad. 2013;26(1):31–4.
[23] Martín-Timón I. Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? Vol. 5, World Journal of Diabetes. 2014. p. 444.
[24] Hurst RT, Mohler ER, Rembold CM, Najjar SS, Stein JH, Kendall CB, et al. Use of Carotid Ultrasound to Identify Subclinical Vascular Disease and Evaluate Cardiovascular Disease Risk: A Consensus Statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force Endorsed by the Society for Vascular. J Am Soc Echocardiogr. 2008;21(2):93–111.
[25] Kota SK, Mahapatra GB, Kota SK, Naveed S, Tripathy PR, Jammula S, et al. Carotid intima media thickness in type 2 diabetes mellitus with ischemic stroke. Indian J Endocrinol Metab [Internet]. 2013 Jul [cited 2019 Jun 3];17(4):716–22.
[26] Toth PP. Subclinical atherosclerosis: What it is, what it means and what we can do about it. Vol. 62, International Journal of Clinical Practice. 2008. p. 1246–54.
[27]Jaffer FA, O'Donnell CJ, Larson MG, Chan SK, Kissinger K V., Kupka MJ, et al. Age and Sex Distribution of Subclinical Aortic Atherosclerosis. Arterioscler Thromb Vasc Biol [Internet]. 2002 May [cited 2019 Mar 4];22(5):849–54.
[28] Roglic G. Global report on diabetes. World Heal Organ [Internet]. 2014;58(12):1–88.
[29] Long-Term Effects of Intensive Glucose Lowering on Cardiovascular Outcomes. N Engl J Med [Internet]. 2011 Mar 3 [cited 2019 Mar 4];364(9):818–28.
[30] Sabbag CRD, Bosch MA, Presti C, Porta RMP, Casella IB, Yamazaki Y. A practical protocol to measure common carotid artery intima-media thickness. Vol. 63, Clinics. 2008.
[31] Chu B, Hatsukami TS, Polissar NL, Zhao X-Q, Kraiss LW, Parker DL, et al. Determination of Carotid Artery Atherosclerotic Lesion Type and Distribution in Hypercholesterolemic Patients With Moderate Carotid Stenosis Using Noninvasive Magnetic Resonance Imaging. Stroke [Internet]. 2004 Nov [cited 2019 Mar 4];35(11):2444–8.
[32] Gleissner CA, Galkina E, Nadler JL, Ley K. Mechanisms by which diabetes increases cardiovascular disease. Vol. 4, Drug Discovery Today: Disease Mechanisms. 2007. p. 131–40.

[33] . Kondos GT, Hoff JA, Sevrukov A, Daviglus ML, Garside DB, Devries SS, et al. Electron-Beam Tomography Coronary Artery Calcium and Cardiac Events. Circulation [Internet]. 2003 May 27 [cited 2019 Mar 4];107(20):2571–6.
[34] Hristova K. The Role of Noninvasive Imaging for Detection High Risk Patients with Subclinical Atherosclerosis. Arch Clin Hypertens. 2018;2:004-012.
[35] DeMaria AN, Narula J, Mahmud E, Tsimikas S. Imaging Vulnerable Plaque by Ultrasound. J Am Coll Cardiol [Internet]. 2006 Apr [cited 2019 Mar 4];47(8):C32–9.

[36] Grobbee De, Bots ML. Carotid artery intima-media thickness as an indicator of generalized atherosclerosis. J Intern Med [Internet]. 1994 Nov [cited 2019 Mar 4];236(5):567–73.
[37] Simon A, Chironi G, Levenson J. Performance of Subclinical Arterial Disease Detection as a Screening Test for Coronary Heart Disease. Hypertension [Internet]. 2006 Sep [cited 2019 Mar 4];48(3):392–6.
[38] Okeahialam BN, Alonge BA, Pam SD, Puepet FH. Carotid Intima Media Thickness as a Measure of Cardiovascular Disease Burden in Nigerian Africans with Hypertension and Diabetes Mellitus. Int J Vasc Med. 2011;2011:1–4.
[39] Darabian S, Hormuz M, Latif MA, Pahlevan S, Budoff MJ. The Role of Carotid Intimal Thickness Testing and Risk Prediction in the Development of Coronary Atherosclerosis. Curr Atheroscler Rep [Internet]. 2013 Mar 18 [cited 2019 Mar 4];15(3):306.
[40] de Groot E, Jukema JW, Montauban van Swijndregt AD, Zwinderman AH, Ackerstaff RGA, van der Steen AFW, et al. B-Mode Ultrasound Assessment of Pravastatin Treatment Effect on Carotid and Femoral Artery Walls and Its Correlations With Coronary Arteriographic Findings: A Report of the Regression Growth Evaluation Statin Study (REGRESS). J Am Coll Cardiol [Internet]. 1998 Jun [cited 2019 Mar 4];31(7):1561–7.
[41] Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: The Rotterdam Study. Circulation. 1997;96(5):1432–7.
[42] O'Leary DH, Kronmal RA, Polak JF, Manolio TA, Burke GL, Wolfson SK. Carotid-Artery Intima and Media Thickness as a Risk Factor for Myocardial Infarction and Stroke in Older Adults. N Engl J Med. 2002;340(1):14–22..
[43] DeLuca AJ, Kaplan S, Aronow WS, Sandhu R, Butt A, Akoybyan A, et al. Comparison of Prevalence of Unrecognized Myocardial Infarction and of Silent Myocardial Ischemia Detected by a Treadmill Exercise Sestamibi Stress Test in Patients With Versus Without Diabetes Mellitus. Am J Cardiol [Internet]. 2006 Oct [cited 2019 Mar 4];98(8):1045–6.
[44] Malik S, Budoff MJ, Katz R, Blumenthal RS, Bertoni AG, Nasir K, et al. Impact of Subclinical Atherosclerosis on Cardiovascular Disease Events in Individuals With Metabolic Syndrome and Diabetes: The Multi-Ethnic Study of Atherosclerosis. Diabetes Care [Internet]. 2011 Oct 1 [cited 2019 Mar 4];34(10):2285–90.
[45] Sabbag CRD, Bosch MA, Presti C, Porta RMP, Casella IB, Yamazaki Y. A practical protocol to measure common carotid artery intima-media thickness. Vol. 63, Clinics. 2008.

[46] Chyun DA, Young LH. Diabetes Mellitus and Cardiovascular Disease. Nurs Clin North Am. 2006;41(4):681–95.

[47] Touboul P-J, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, et al. Mannheim Carotid Intima-Media Thickness Consensus (2004–2006). Cerebrovasc Dis [Internet]. 2007 [cited 2019 Mar 4];23(1):75–80.

[48] National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002; 106: 3143 - 3421.

[49] Hong E-G, Ohn JH, Lee SJ, Kwon HS, Kim SG, Kim DJ, et al. Clinical implications of carotid artery intima media thickness assessment on cardiovascular risk stratification in hyperlipidemic Korean adults with diabetes: the ALTO study. BMC Cardiovasc Disord [Internet]. 2015 Dec 6 [cited 2019 May 26];15(1):114.

[50] Khaleeli E, Peters SR, Bobrowsky K, Oudiz RJ, Ko JY, Budoff MJ. Diabetes and the associated incidence of subclinical atherosclerosis and coronary artery disease: Implications for management. Am Heart J [Internet]. 2001 Apr [cited 2019 May 26];141(4):637–44.

[51] Perez HA, Garcia NH, Spence JD, Armando LJ. Adding carotid total plaque area to the Framingham risk score improves cardiovascular risk classification. Arch Med Sci [Internet]. 2016 [cited 2019 May 24];3:513–20.

[52] Junyent M, Zambón D, Gilabert R, Núñez I, Cofán M, Ros E. Carotid atherosclerosis and vascular age in the assessment of coronary heart disease risk beyond the Framingham Risk Score. Atherosclerosis [Internet]. 2008 Feb [cited 2019 May 26];196(2):803–9.

[53] Joshi MD, Ayah R, Njau EK, Wanjiru R, Kayima JK, Njeru EK, et al. Prevalence of hypertension and associated cardiovascular risk factors in an urban slum in Nairobi, Kenya: A population-based survey. BMC Public Health. 2014;14:1177.

[54] Martín-Timón I, Sevillano-Collantes C, Segura-Galindo A, Del Cañizo-Gómez FJ. Type 2 diabetes and cardiovascular disease: Have all risk factors the same strength? World J Diabetes [Internet]. 2014 Aug 15 [cited 2019 May 27];5(4):444–70.

[55] Mamudu HM, Alamian A, Paul T, Subedi P, Wang L, Jones A, et al. Diabetes, subclinical atherosclerosis and multiple cardiovascular risk factors in hard-to-reach asymptomatic patients. Diabetes Vasc Dis Res [Internet]. 2018 Nov 16 [cited 2019 May 28];15(6):519–27.

[56] Hegazi R a F, Kim S-T, W. ER, H. KL, Steven B, Monica Y, et al. Relationship of adiposity to subclinical atherosclerosis in obese patients with type 2 diabetes. Obes Res. 2003;11(12):1597–605.

[57] Kawase Ishihara K, Kokubo Y, Yokota C, Hida E, Miyata T, Toyoda K, et al. Effect of plasma fibrinogen, high-sensitive C-reactive protein, and cigarette smoking on carotid atherosclerosis: the Suita study. J Stroke Cerebrovasc Dis. 2015.

[58]Fang N, Han W, Gong D, Zou Chen Z, Fan Y. Atorvastatin Treatment for Carotid Intima-Media Thickness in Chinese Patients With Type 2 Diabetes: A Meta-Analysis. Medicine (Baltimore) [Internet]. 2015 Nov [cited 2019 May 27];94(44):e1920.

[59] THE ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY: DESIGN AND OBJECTIVES. Am J Epidemiol [Internet]. 1989 Apr 1 [cited 2019 May 28];129(4):687–702

APPENDICES

APPENDIX 1: CONSENT FORM (ENGLISH)

CONSENT FORM

Research title: subclinical atherosclerosis in diabetic patients in KNH diabetic outpatient clinic

This consent form consists of the following:

- An information sheet
- Consent certificate
- Statement by the researcher/ research assistant

Information sheet

Introduction

Diabetes mellitus is a disease that affects various aspects of the human body. One of the areas affected are the blood vessels. Diabetes, by various mechanisms, can lead to narrowing and blockage of the blood vessels which can in turn lead to complications such as stroke and heart attack, among others.

Study purpose

No studies have been done in Kenya to determine the extent of blood vessel problems in diabetics. This study will look at the neck vessels of the participants to determine the degree of narrowing of these vessels which will assist us to gain more knowledge and treat patients better.

Risks and benefits

Risk of complications with carotid ultrasound are minimal as the procedure is non-invasive, does not emit ionizing radiation and has been deemed safe by extensive research in the field of ultrasonography. Also of note is that no laboratory tests will be done for the purpose of this study and as such no blood samples will be taken from the participants.

As a patient with diabetes, this study will be able to tell if there is a problem with your blood vessels. If such a problem is detected your doctor will talk to you about the risks involved and how to avoid complications of vascular disease. The ultrasound examination done will be at no added cost to the patient.

Study Procedures

Carotid ultrasound requires a patient to lie down on a couch with their neck extended. The neck vessels will be examined by applying a water based gel and placing an ultrasound probe on both sides of the neck intermittently. Thickness of the vessel walls will be taken electronically and results explained to the patient and conveyed to the principal physician at the diabetic clinic. The procedure has no known side effects and does not submit the patient to ionizing radiation. There is no recovery period required and the patient is free to leave as soon as the procedure is complete

Voluntariness of participation

Participation in this study is entirely voluntary and you will not be denied medical care in case you refuse to participate. If you do decide to take part you shall be expected to sign the underlying consent form. You may withdraw from participating in the study at any time with no consequence whatsoever.

Confidentiality

All information will be treated with confidentiality and any relevant medical information regarding the results of the ultrasounds done and the data collected will be accessible only to persons authorised to handle it. This will include the researcher, their supervisors and the patient's primary care-giver, if so required.

All patients personal information collected will be destroyed at the end of the study.

No records of names of the patients/ relatives will be kept in the data collection.

Compensation

No compensation, financial or otherwise, will be offered to the participants. Neither will any preferential treatment, gift or reward, be awarded to the participants during or after the duration of the study.

Contact information

Should you require any further clarification regarding the study please feel free to contact the principal researcher:

Dr Husein M. Tayabali,

Radiology resident, University of Nairobi.

Telephone Number: 0738644800

Supervisor:

Dr. M. Mutala,

Department of Diagnostic Imaging and Radiation Medicine,

P. O. Box 19676-00202, Nairobi.

E-mail: musilamutala@gmail.com

For queries concerning your rights as a research participant you may contact the Kenyatta National Hospital Ethics and Research Committee. It is the mandate of this committee to protect you, if you chose to participate, from harm.

KNH-UoN Ethics and Research Committee,

P.O. Box 19676-00202 OR P.O Box 20723-00202, Nairobi.

E-mail: uonknh_erc@uonbi.ac.ke

Consent form

I, the undersigned, have read and fully understood the explanation given to me regarding the above mentioned study. I have been given the opportunity to ask questions which have been answered satisfactorily by the investigators.

I understand that my participation is voluntary and that I have not been forced t take part in the study and that I can decline of my own accord without my medical care being affected.

I understand that I will not receive any form of remuneration or preferential treatment for taking part in the study.

I understand that my personal information will be kept confidential, but that any pertinent medical information obtained from my ultrasound scans and the data collected therefrom may be accessible to the principal investigator and their supervisors.

I hereby consent to my participation in this study.

SIGNED: …… (Patient)

Date: …….

Unique Patient ID: …….....

SIGNED: ……. (Witness)

Statement by the researcher

I hereby confirm that I have adequately explained the contents of the information sheet to the participant; and that they understand the voluntary nature of their participation in the study as well the confidentiality with which their information will be treated; and their right to refuse or withdraw from the study without fear of compromise to their quality of care.

Name……..

Signature……

Date……....

APPENDIX 2: CONSENT FORM (SWAHILI)

Fomu ya idhini ya kushiriki katika utafiti

Mada: Kukusanyika mafuta katika mishipa ya shingo ya wagonjwa waliyoathiriwa na kisukari katika kliniki hospitalini Kenyatta.

Fomu hili lina sehemu tatu:

- Maelezo kwa ufupi kuhusu utafiti
- Fomu ya makubaliano ya kushiriki katika utafiti
- Dhibitisho la mtafiti/mtafiti msaidizi

Maelezo kwa ufupi kuhusu utafiti

Utangulizi

Ugonjwa wa kisukari unaweza kuathiri viungo tofauti vya mwili wa binadamu. Moja ya viungo hivyo ni mishipa ya damu. Ugonjwa wa kisukari unasababisha kufungwa ama kuwa nyembamba

kwa hayo mishipa kwa namna mbali mbali na huenda ikaleta shida zingine mwilini kama kiharusi ama mshtuko wa moyo.

Madhumini ya utafiti

Nchini Kenya, tafiti za kuchunguza matatizo ya mishipa ya damu kwa wagonjwa walioambukizwa na kisukari hazijafanywa. Kwa hivyo, utafiti huu unalenga kuangalia suala hili, na itatusaidia kuweka mikakati ya mapema kutambua matatizo ya mishipa ya damu ili kuwaelimisha na kutibu wagonjwa wetu bora zaidi.

Madhara na faida

Athari ya kushiriki katika utafiti huu ni chache sana. Hakuna uchungu wowote utasikia ukipigwa picha ya mishipa ya damu ya shingo, na hakuna mionzi ya x ray katika picha hii. Mbali na hayo, hakuna uchunguzi wa damu utafanywa katika utafiti huu.

Kama mgonjwa wa kisukari, utafiti huu utaweza kutambua kama kuna shida na mishipa yako ya damu, hususan yale ya shingo. Shida ikipatikana, daktari wako atakuelezea kuhusu jinsi ya kujikinga na matatizo zinazosababishwa na ugonjwa za mishipa za damu.

Uchunguzi utaofanywa na picha ya ultrasound hautakuwa na malipo yoyote.

Utaratibu wa Mafunzo

Picha ya Mshipa inahitaji mgonjwa kulala chini kwa kitanda na shingo kupanuliwa. Mishipa ya shingo vitafuatiwa kwa kutumia gel ya maji na kuweka Kifaa cha kufanya utafiti pande zote mbili za shingo upande mmoja baada ya mwingine. Uzani wa kuta za mishipa zitachukuliwa na matokeo yataeelezwa kwa mgonjwa na kupelekwa kwa daktari mkuu katika kliniki ya kisukari.
Hakuna mda utaohitajika mgonjwa kujikinga, na ako huru kwenda mara hiyi hiyo akisha maliziwa kipimo.

Kujitolea kwa mshiriki

Ni hiari yako kuingia katikia utafiti huu. Ukiamua kutojiunga hutokatalizwa matibabu yako ya kawaida katika hospitali. Ukiamua kujiunga na utafiti huu, utapewa fomu hii ya maelezo na kusaini kartasi ya makubaliano. Ukiingia utafiti huu, una uhuru wa kutoka wakati wowote bila athari yoyote.

Siri ya taarifa iliyokusanywa

Taarifa yote itakayokusanywa itawekwa siri na taarifa yoyote muhimu kuhusu matokeo ya picha zilizopigwa yataangaliwa na watafiti waliohusika pekee. Habari yote ya kibinafsi kuhusu mshiriki yatafutwa mwisho wa utafiti. Hakuna rekodi ya majina ya wagonjwa yatawekwa katika ukusanyaji wa takwimu .

Fidia

Hakuna fidia ya kifedha, zawadi ama aina yoyote ingine ambayo washiriki watapewa ama kupewa huduma kwa upendeleo aidha wakati wa ama baada ya utafiti.

Maelezo ya mawasiliano

Ukihitaji ufafanuzi zaidi kuhusu utafiti huu tafadhali wasiliana na mtafiti mkuu:

Dkt. Husein M. Tayabali,

Idara ya Radiolojia, Chuo Kikuu cha Nairobi.

Nambari ya Simu: 0738644800

Msimamizi:

Dkt. M. Mutala,

Idara ya Radiolojia, Chuo Kikuu cha Nairobi,

Sanduku La Posta: 19676-00202, Nairobi.

Barua Pepe: musilamutala@gmail.com

Ukiwa na maswali yoyote kuhusu haki zako kama mshiriki katika utafiti huu unaweza wasiliana na KNH - UON Maadili na Kamati ya Utafiti. Ni jukumu la kamati hili kukulinda kutoka na madhara ukijchagua kushiriki katikia utafiti huu.

KNH - UON Maadili na Kamati ya Utafiti,

S.L.P 19676-00202 AMA 20723-00202 Nairobi, Kenya.

Barua Pepe: uonknh_erc@uonbi.ac.ke

Fomu ya idhini

Mimi, niliyepiga sahihi hapa chini, nimesoma na kuelewa maelezo niliyepewa kuhusu utafiti huu. Nimepewa muda wa kuuliza maswali ambayo yamejibiwa kwa kuridhisha na watafiti.

Naelewa kwamba ushirikiano wangu ni kwa hiari yangu na sijalazimishwa kushiriki katika utafiti huu na kuwa naweza kukataa kushiriki kwa nia yangu mwenyewe bila matibabu yangu kuathiriwa.

Naelewa kwamba sitopewa malipo yoyote wala huduma kwa mapendeleo nikiamua kushiriki katika utafiti huu.

Naelewa kwamba taarifa yangu ya kibinafsi itawekwa siri lakini hata hivyo taarifa kuhusu matokeo ya uchunguzi itayokusanywa wakati wa utafiti kutokana na picha itaweza kutumika na kuchambuliwa na mtafiti mkuu na wasimamizi wake pindi itakavyohitajika.

Mimi natoa ridhaa ya kushiriki katika utafiti huu.

Sahihi..... (Mshiriki)

Tarehe.....

Nambari ya Mshiriki .....

Sahihi: ..... (Shahidi)

Kauli la mtafiti

Mimi na thibitisha kwamba nimeelezea mshiriki yaliyomo katika katikia fomu ya idhini na kuwa wanaelewa kwamba ushiriki yao ni kwa hiari yao na ya kuwa taarifa yao itawekwa siri na wana haki ya kukataa au kujitoa kwa utafiti huu bila kuhofia kuwa matibabu yao ya kawaida itaathirika

Jina.....

Sahihi.....

Tarehe.....

APPENDIX 3: QUESTIONNAIRE

QUESTIONNAIRE

Date: …… Study No: ……...

Date of Birth: ……... Age: ……

Year of Diagnosis of Diabetes: ……

A. DEMOGRAPHICS

1. Gender

□ Male □ Female

2. Marital status

□ Single □ Married □ Divorced □ Widowed
□ Separated

3.Residence ……

4. Occupation

□ Self-employed
□ Employed
□ Unemployed
□ Retired
□ Student

5. Education Level

□ None
□ Primary School
□ Secondary School

- Tertiary Level
- Other (specify) ……

B. CHRONIC ILLNESSES

6. Hypertension

- Yes
- No

Duration (Years) ……

C. PAST MEDICAL HISTORY

7. Have you ever had any of the following? (Tick where appropriate)

- Heart attack
- Angina pectoris (sudden onset chest pain)
- Coronary bypass surgery (surgery to unblock the vessels of the heart)
- Transient ischemic attacks (short term strokes with complete recovery)
- Blockage of the blood vessels of the legs
- Stroke

D. FAMILY HISTORY

8. Did or does any of your relatives suffer from hypertension?

- Yes
- No

9. Did any of your first-degree relatives (father, mother, brothers, sisters or children) suffer from heart attack, stroke or sudden death?

- Yes
- No

E. SMOKING

10. Do you smoke cigarettes now?

- Yes
- No

11. On average how many cigarettes do you smoke per day? …….. cigarettes/day.

12. Did you ever smoke cigarettes regularly in the past?

- Yes
- No

13. For how many years have you been smoking cigarettes? ……years.

F. ALCOHOL INTAKE

14. Do you drink alcohol?

- Yes
- No

G. CURRENT MEDICATIONS

Are you currently on any of the following medications?

15. Blood pressure lowering drugs?

- Yes
- No

16. Lipid lowering drugs?

- Yes
- No

17. Antiplatelet drugs? (Aspirin/Clopidogrel)

- Yes
- No

H. PHYSICAL EXAMINATION

18. Height (m) ……..

19. Weight (kg) ……..

20. BMI (kg/m2) ……

21. Waist Circumference (cm) ……

22. Hip Circumference (cm) …….

23. Waist/Hip Ratio ……

24. Blood Pressure ……