POSTMORTEM ANALYSIS OF CORONARY ATHEROSCLEROSIS AMONG YOUTH DYING OF UNNATURAL CAUSES

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EXPRESSION OF DECLARATION

I hereby declare that this dissertation is my original work under the guidance of the supervisors listed below and has not been submitted to the University of Nairobi or any other higher learning institution for review and approval.

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ACRONYMS

AHA	American Heart Association
ARV	Antiretroviral Therapy
BMI	Body Mass Index
CIMT	Carotid Intima Media Thickness
CVA	Cerebrovascular Accident
CVD	Cardiovascular Disease
ECM	Extracellular Matrix
ERC	Ethical Research Committee
HCL	Hydrochloric Acid
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
H&E	Haematoxylin and Eosin
KNH	Kenyatta National Hospital
LAD	Left Anterior Descending Artery
LCA	Left Circumflex Artery
LDL	Low Density Lipoprotein
MI	Myocardial Infarction
MT	Masson's Trichome
NCD	Non-Communicable Disease
PDAY	Pathobiological Determinants of Atherosclerosis in Youth
PI	Principle Investigator
RCA	Right Coronary Artery
RTA	Road Traffic Accident

SMC	Smooth Muscle Cells
SOP	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences
TG	Triglyceride
UON	University of Nairobi
VLDL	Very Low-Density Lipoprotein
WHO	World Health Organisation
WHR	Waist to Height Ratio

DEFINITION OF TERMS

Atherosclerosis: Chronic inflammatory progressive disease afflicting the arteries associated with fibrosis and deposition of lipids on the vessel walls.

Cause of Death: Official determination that resulted in death of a human.

Decedent: An individual who has died

Youth: Young people belonging to age group of 15 to 35 years (Africa Union).

Waist Height Ratio: Measure of obesity derived from dividing waist by height in the same units

ABSTRACT

Background: Cardiovascular diseases (CVDs) are the most common causes of non-communicable diseases (NCDs) in Kenya with atherosclerosis contributing a major portion. There is a steady increase in its prevalence in the youth with commencement appreciated as early as infancy. Predisposing factors to atherosclerotic lesions such as alcohol consumption and smoking are on the rise among the youth. There is paucity of data on prevalence and patterns of atherosclerosis among this age group in our setting. Postmortem provides the gold standard for assessing atherosclerosis.

Objective: To evaluate through postmortem the presence and histologic type of coronary atherosclerosis among youth who have died from unnatural causes and to assess the correlation between coronary atherosclerosis and known risk factors.

Study Design: Descriptive cross-sectional study

Study Area: Kenyatta National Hospital (KNH) Mortuary and City Mortuary, Kenya. Tissue processing was done at the University of Nairobi Histopathology Laboratory.

Study Population: Decedents from unnatural deaths aged between 18 and 35 years.

Materials and Methods: Study was conducted on 95 decedents. Data on risk factors was obtained from the next of kin of the decedent using a structured questionnaire. Anthropometric measures were recorded. Postmortems were done and the hearts excised, weighed and fixed in formalin for one week. The 3 coronary arteries were systematically dissected to check for gross changes and excised. They were processed and stained with H&E (Haematoxylin and Eosin) and MT (Masson's Trichrome) and evaluated for atherosclerotic lesions which were graded according to the American Heart Association (AHA) classification of atherosclerosis.

Results: The study included 79 male decedents and 16 female decedents. Amongst them 51(53.7%) had presence of coronary atherosclerosis. Of these cases, 82.4% (n=51) were males and 17.6% (n=51) were females. There was no statistically significant relationship between atherosclerosis and the assessed risk factors. Left

circumflex artery was the most affected with a prevalence of 41.1% (n=95). The most common type of atherosclerosis was Type 2 followed by Type 3. Single vessel atherosclerosis was the most prevalent at 32.6% (n=95) of the cases.

Conclusions: There is presence of coronary atherosclerotic lesions in young decedents in our setting with a high prevalence noted. There was no significant relationship determined between the risk factors assessed and coronary atherosclerosis.

Recommendations: Future studies that target a wider study population that incorporate epidemiologic data, detailed medical records and cardiovascular biomarkers could be done to further aid in analysis of atherosclerosis in the young adults in our setting.

1.0 CHAPTER ONE: INTRODUCTION

Atherosclerosis is a disease of medium and large arteries with common manifestation clinically as ischemic heart disease and stroke. The term atherosclerosis is a Greek word meaning wax hardening which corresponds to the atherosclerotic plaque necrotic center which is covered by a fibrous layer. Atherosclerosis is the primary etiology of cardiovascular diseases (CVD) which are a common cause of death globally and claims millions of lives every year. More people die annually from CVD than from any other cause, with an estimated 17.5 million deaths in 2012 which contributed to 46% of all non-communicable disease (NCD) deaths (1). Initially, atherosclerosis was constricted to a disease of developed countries where it is the leading cause of death and attributed to sedentary lifestyle and unhealthy diet but the prevalence in developing countries as noted is on a steady rise (2). The developing world is quickly catching up with this trend and it is estimated by 2020 CVDs will be the most common cause of morbidity and mortality in developing nations (3).

Cardiovascular diseases are the most common causes of NCDs in Kenya (4). There has been a steady rise in cardiovascular related mortalities which contributed to 8% of the total deaths in the country in 2014 (4). Atherosclerotic diseases are the most common affliction of the coronary arteries. It eventually leads to various complications like myocardial infarction, stroke and peripheral vascular disease. A much in-depth insight into atherosclerosis can aid in curbing these serious effects globally (5).

Atherosclerosis has typically been associated with the elderly but various studies have shown it commences from early childhood (6). Predisposing factors such as smoking, sedentary lifestyle, a high fat diet and excessive alcohol intake are on the rise especially among the young population further increasing the risk of atherosclerosis (7). Due to absence of symptoms in this age group, there is possibility of undetected cases of atherosclerosis which is usually termed as subclinical atherosclerosis. The prevalence of atherosclerosis in this age group is usually indeterminate or underestimated in many parts of the world and especially in developing countries where the surge in incidence has been noted (3).

There is paucity of local data on prevalence among the youth with resultant lack of aggressive methods of arresting atherosclerosis in its early stage (8). However, it can be postulated due to the current change of lifestyle there is a high prevalence. Increased public education, promotion of healthy lifestyle, early screening and treatment have been shown to reduce prevalence of atherosclerosis and its associated complications (9).

Postmortems provide an excellent source of information on atherosclerosis in terms of determining its prevalence, grading and pattern due to its increased sensitivity as compared to other modalities like biochemical testing and imaging: also, due to the fact that invasive means of assessing atherosclerosis is impracticable in our setting in living subjects due to limited availability and associated high costs.

This study therefore aimed, through postmortem, to evaluate the coronary arteries for atherosclerosis and if present, determine its severity among apparently healthy young decedents who have died from unnatural causes enabling the detection of prevalence of coronary atherosclerosis and determine any possible association with known risk factors. The outcome of this study will aid in advocacy for management of atherosclerosis and associated risk factors for the development of atherosclerosis in young adulthood.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Pathophysiology of Atherosclerosis

Atherosclerosis is a chronic progressive disease associated with fibrosis and deposition of lipids on the vessel wall (10). It is a complex multifactorial progressive disease with a major role played by inflammation (11). Postmortems done in children have shown that atherosclerosis begins at an early age with the commencement of an initial lesion which is the presence of lipid laden macrophages (foam cells) in the sub endothelial layer (5,7). It undergoes various stages of which the early stages are deemed to be reversible if detected promptly (10,11). The presence of fatty streaks acts as a precursor of advanced lesions where there is accumulation of lipid rich necrotic debris, smooth muscle cells and extracellular matrix with development of plaques which can undergo haemorrhage, ulceration or calcification (10,11). They can result in vessel occlusion once they rupture or erode causing thrombosis and consequent myocardial infarction or stroke in affected vessels (5).

Various researches are ongoing to determine the molecular mechanism of atherosclerosis since it has been determined it is not merely a disease of excessive lipids in the body (11). Studies have shown there is a central role of inflammatory cells and endothelium in the development of atherosclerosis (10). A major initiator of atherosclerosis process is LDL accumulation at the sub-endothelium which is notable when there is increased plasma LDL (5). It is believed that it undergoes oxidation, lipolysis and other degenerative processes which triggers inflammatory process at the intima (5,11). Other factors are also likely to modulate inflammation e.g. diabetes, infections and haemodynamic factors (10, 12). There is consequent recruitment of lymphocytes and monocytes, which will transform to macrophages, to the arterial wall. Foam cells are a consequence of uptake of modified LDL by these macrophages (5). Continuous macrophage secretion of multiple cytokines and growth factors results in migration, proliferation and accumulation of SMC with extracellular matrix production leading to development of fibrous plaques (11, 12). Vulnerability of these plaques can result in their rupture or erosion leading to acute coronary events.

2.2 Factors Associated with Development of Atherosclerosis

2.2.1 Atherosclerosis in Youth

Youth in our setting is all persons aged between 15 and 35 years according to the African Union (13). Even though cardiovascular diseases clinically manifest in adulthood, atherosclerosis is a progressive disease which begins in childhood as determined by various autopsy studies (14). Postmortems done on youth have determined a strong relationship between subclinical atherosclerosis and cardiovascular risk factors (15). Reduction in healthy lifestyle factors during early formative years is associated with the risk of developing subclinical atherosclerosis with consequent progression in middle age (9). These factors include presence of obesity/overweight, physical inactivity, smoking, high alcohol consumption and unbalanced diet. Most young people progress to adulthood with at least one of the unhealthy behaviors (9). Therefore, promotion of lifestyle changes at an earlier age is essential to aid in curbing atherosclerosis.

2.2.2 Gender

The risk for developing atherosclerosis is higher in males than females due to increased engagement in traditional risk factors like alcohol intake and smoking but there is increasing prevalence in women as a consequence of dietary trend and smoking (16).Coronary atherosclerosis has been found to progress more rapidly in young men than in young women (17). But in a study done by Oyama et al they found increased aortic atherosclerosis in women more than men with increased plaque volume greater in women (18). Therefore, gender on its own is not a conclusive risk factor for atherosclerosis.

2.2.3 Alcohol

Alcohol has both beneficial and harmful effects in relation to atherosclerosis on CVDs (19). Light to moderate alcohol intake has been shown by various epidemiological studies to reduce the risk of cardiovascular diseases (19). Alcohol is associated with an increased levels of HDL cholesterol and decreased fibrinogen levels. This is thought to be a protective effect if alcohol is consumed in light to moderate amount (19). Heavy consumption of alcohol on the contrary is associated with increased risk of development of atherosclerosis (19). Study done by

Mukamal et al showed CIMT, as a measure of subclinical atherosclerosis, was highest among heavy drinkers who drank more than 14 drinks per week (20). Wine is thought to be a protective factor but no conclusive association has been found between alcohol beverage type and atherosclerosis (20).

2.2.4 Smoking

Smoking is one of the leading aetiological factors in cardiovascular disease (21). There is increased smoking among the youth and those who commenced smoking during teenage years are more likely to continue into adulthood (21). The pathogenetic mechanism of smoking related atherosclerosis is largely unknown but smoking causes chronic inflammation, thrombosis and LDL oxidation. Current smoking has been associated with risk and progression of coronary atherosclerosis according to autopsy studies (22). Wissler et al demonstrated a higher prevalence of advanced atherosclerotic plaques in the coronary arteries in smokers as compared to nonsmokers (22). There is increased risk of coronary atherosclerosis associated with smoking with a projected risk of 3 times as compared to non-smokers (23). Cessation of smoking has been shown to reduce the progression of atherosclerosis generally therefore it is an important preventive measure in reducing morbidity and mortality (21).

2.2.5 Diabetes Mellitus

Diabetes mellitus is one of the known traditional risk factors associated with atherosclerosis. Diabetic patients have a 2 to 4 fold risk for developing CVDs and 80% of the mortality among them is due to CVDs (24). Both type 1 and type 2 diabetes show a similar atherosclerotic lesion with an increased necrotic core size and decreased fibrotic cap size (24). But despite this, type 2 diabetes shows marked atherosclerotic plaque burden as compared to type 1 diabetes (24). Also type 2 diabetes is associated with multiple atherogenic factors like obesity, hyperlipidemia and hypertension therefore increasing the atherosclerotic plaque burden (24,25). In type 1 diabetes which is more prevalent in children, hyperglycemia alone without these atherogenic factors is thought to accelerate atherosclerosis (24).

Individuals with impaired fasting glucose and impaired glucose tolerance have been shown to have more advanced coronary atherosclerosis than non-diabetic individuals and to have comparable lesions with patients with diabetes (25). Therefore, apart from diabetes, prediabetic state could be a risk factor for the development of atherosclerosis.

2.2.6 Hypertension

Hypertension plays a prominent role as a modifiable risk factor for atherosclerosis. Hypertension seems to contribute to the development of fibrous plaque. Angiotensin II, which plays a role in raised blood pressure, directly increases SMC proliferation and ECM production (10).

According to the PDAY study, they found a mean arterial pressure of more than 110mmHg was associated with presence of extensive raised lesions in both the abdominal aorta and the right coronary artery (26). Assessment of participants in the Framingham Heart Study Offspring cohort showed increased aortic plaque burdens in hypertensive patients as compared to normotensive patients (27).

2.2.7 Obesity and Dyslipidaemia

Obesity is a known risk factor for development of cardiovascular diseases. There is increasing prevalence of obesity beginning in childhood (9). It is thought to play a role in atherosclerosis by increasing systemic proinflammatory cytokines and decreasing protective factors e.g. adiponectin and this is more marked in abdominal obesity (24). Lipid levels are a known predictor of coronary artery disease. The ratio of TG/HDL and HDL has a protective effect from atherosclerosis while non-HDL play a central role in progression of atherosclerosis in tandem with other risk factors. Atherogenic dyslipidaemia consists of high triglyceride and VLDL levels and reduced HDL cholesterol levels. The PDAY study found a correlation between levels of non HDL and extent of fatty streaks and raised lesions in the right coronary artery and aorta of youth between 15 and 34 years of age (28). The Bogalusa Heart Study found out levels of LDL and BMI measured during childhood predict CIMT which is used as a marker for subclinical atherosclerosis in young adulthood (15). Maternal hypercholesterolemia during pregnancy has been postulated to be one of the pathogenic events in the foetus which leads to development of atherosclerotic lesions (16). In a local study to assess presence of

atherosclerosis in the Maasai Tribe, it was found that there was low levels of cholesterol in moran subjects between the age of 12 and 30 years and this was attributed to their high protein diet and increased level of activity and physical fitness (29).

2.2.8 Other Risk Factors

With increasing pathophysiological studies on atherosclerosis, cellular and molecular interactions have been postulated as a cause of atherogenesis and this has resulted in the shift of focus to new emerging risk factors to further broaden the pathogenetic mechanism. These include infections and genetic factors.

2.2.8.1 Infections

There is an increasing evidence of association between infectious pathogens and development of atherosclerosis. Antibodies to these pathogens have been found to be present in individuals with atherosclerosis and some have actually been demonstrated in atherosclerotic lesions (30). The main mediator for this process is chronic inflammation. Microbes activate T cells, macrophages and mast cells which results in release of cytokines e.g. interferon – gamma which can result in atherosclerotic plaque instability and metalloproteinases and cysteine proteases release which directly destroy collagen thereby precipitating plaque formation (10). There are many theories advanced to try to associate these infections with the development and progression of atherosclerosis but none so far has been conclusively proven.

a. HIV

HIV is associated with chronic inflammation and immune activation which can increase the risk of developing atherosclerosis. Also, the use of ARVs especially protease inhibitors is associated with dyslipidaemias which can enhance further progression of atherosclerosis. Among HIV positive patients, cardiovascular diseases are the second most common cause of death (31). In a study done by Ssinabulya et al in Uganda, they analysed carotid intima media thickness via ultrasound as a marker of subclinical atherosclerosis. Out of 186 patients, they found 18% with subclinical atherosclerosis out of whom 56% were on ARVs (32).

b. *Chlamydia Pneumoniae*

It has been postulated that chlamydia pneumoniae is a risk factor for early atherosclerosis. In a study done by M. Player et al they found *C.pneumoniae* antibodies presence is related to the progress of coronary artery calcification which is a marker for subclinical atherosclerosis (30). They also suggested that smoking enhances the association between *C.pneumoniae* and coronary artery calcification. It has also been suggested that *C. pneumoniae* is a risk factor for atherosclerosis in association with hyperlipidemia (33).

c. Periodontal Pathogens

Periodontitis is thought to play a role in atherosclerosis. Implicated pathogens are *P. gingivalis* and *A. actinomycetemcomitans* which are thought to gain vascular access through bleeding of gums (33). Studies done on animal models inoculated with these organisms have shown both early atherosclerosis formation and accelerated atherosclerosis progression in hyperlipidaemic mice and rabbits (33).

2.2.8.2 Role of Genetics

Genetics plays a major role in susceptibility to development of atherosclerosis. Epidemiological studies have shown its heritability in twins and families with most exceeding 50% (34). Positive history of cardiovascular disease in the family is an independent risk factor for development of atherosclerosis. Children who have parents or grandparents who died due to a cardiovascular event e.g. myocardial infarction, stroke have twice the risk of developing atherosclerosis when they reach middle age (6). The risk of death from CAD increases 5 to 7 fold in first degree relatives of patients with CAD (34). Concordance rate for CAD was found to be 3 fold higher in monozygotic twins than dizygotic twins (34).

The pathogenesis of atherosclerosis is due to both genetic and environmental factors having biologic complex interactions (35). There is involvement of both susceptibility genes and modifier genes. Traditional risk factors linked to the atherosclerotic process e.g. dyslipidaemia, diabetes, hypertension are thought to influence the genes involved in atherosclerosis (35). There are multiple genes which are thought to predispose to atherosclerosis including those involved in inflammation, oxidative stress, lipid metabolism and coagulation (34). Genes associated with lipid metabolism are known to increase plasma levels of pro-

atherogenic lipoproteins which is a major determining factor for the development of atherosclerosis (34).

Studies are ongoing to determine the genes that form the molecular basis of atherosclerosis, predict the risk of premature atherosclerosis development and which will aid in tailoring individual drug therapy for atherosclerosis. Those with a high association include ABCA 1 gene and CYBA gene located on chromosome 16q which have shown promising results as potential genes for atherosclerosis (35).

2.2.8.3 Vascular Site Predisposition

Even though atherosclerosis has systemic predisposing factors, it tends to have a predilection for certain sites of the cardiovascular system. The sites in the human body consistently involved by atherosclerosis are the aorta, coronary and carotid arteries. Atherosclerosis appears in the aorta in the first decade of life, in the coronary arteries in the second decade of life and in the cerebral arteries in the third decade of life (16). According to a study done by Rodriguez et al coronary arteries were the most significantly affected arterial bed (36). In their study, they analysed the circle of Willis, carotids, renal, coronaries and aorta in 186 decedents and found frequency of advanced atherosclerotic lesions in 71 % right coronary artery, 85% left coronary artery, 36% right carotid artery, 25% left carotid artery, 28% circle of Willis, 26% right renal artery, 29% left renal artery and 52% aorta. In a study done by Ogengo et al to determine the presence of atherosclerosis in the tunica adventitia of arteries in black Kenyans, a prevalence of 14.8% was found on the left anterior descending coronary arteries (37).

Haemodynamic factors play an important role in the localisation of atherosclerosis. Vascular beds with low shear stress, oscillating flow and turbulence which cause flow disruption tend to be predisposed to atherosclerotic lesions (38). At the coronary arteries, atheromas occur at sites of bends, branching and bifurcations which could be due to the above factors.

These parameters are thought to play a role in enhancing the progression of atherosclerosis but are not on their own a causative factor. Other parameters like gender, genetic susceptibility, immune status and oxidative stress may each individually influence atherosclerosis in the various sites (39).

2.3 Justification

Atherosclerosis has been noted to be on the rise locally with a paradigm shift of focus from infectious diseases to non-communicable diseases e.g. coronary artery diseases, hypertension and diabetes. Current lifestyle change with exposure to risk factors plays a major role. There is an earlier age of exposure to the risk factors which could play a key role in both the development, progression and determining of the severity of coronary atherosclerosis. Probably, many cases of atherosclerosis are going undetected thereby presenting late when the disease is at an advanced stage contributing to increased morbidity and mortality.

Prevalence and grade of atherosclerotic changes is accurately determined through autopsy studies which are considered the gold standard for investigation of atherosclerosis where the arteries are sampled, processed and analysed. This could consequently be used to correlate the severity of atherosclerosis with the various risk factors and therefore determining the presence of any causal relationship. It is conceptualized that information gathered here can therefore be replicated in the living population to determine future disease burden and contribute to a shift of focus to more studies on atherosclerosis in the youth to adequately address this emerging disease.

2.4 Study Question

Is there increased prevalence of coronary atherosclerosis in the youth? Are there any associated risk factors which might determine this prevalence?

2.5 Broad Objective

- To assess the presence of and classify coronary atherosclerosis among young decedents aged 18-35 years, whose manner of death is unnatural, at KNH and City Mortuaries, Nairobi.

2.6 Specific Objectives

1. To determine the prevalence of atherosclerosis in the coronary arteries among deceased persons aged 18-35 years

2. To classify the atherosclerotic changes and assess distribution in the coronary arteries
3. To determine presence of causal relationship between coronary atherosclerosis and known risk factors - obesity, alcohol use, cigarette smoking, personal history of diabetes and hypertension and family medical history of CVDs.

3.0 CHAPTER THREE: METHODOLOGY

3.1 Study Design

This was a cross sectional descriptive study.

3.2 Study Setting

Kenyatta National Hospital Mortuary and City Mortuary. Processing of the histologic sections was done at UON Histopathology Laboratory.

3.3 Study Population

Decedents between 18 and 35 years of age who had died of unnatural causes in an urban setting (Nairobi).

3.4 Period

The study was carried out between April and July 2017.

3.5 Selection Criteria

3.5.1 Inclusion Criteria

All decedents between the age of 18 and 35 years who had died of unnatural causes principally homicides and accidents admitted into KNH and City Mortuaries during the study period and for whom consent had been obtained from the relatives or guardians.

3.5.2 Exclusion Criteria

1. Unidentified decedents
2. Presence of putrefaction
3. Presence of cardiac birth defects

3.6 Sample Size Determination

The sample size was determined using Kish Leslie's formula for cross sectional studies.

$$N = \frac{p(1-p)Z^2}{d^2}$$

Where:

N = Sample size

Z= Z value (1.96 at 5 % type 1 error [P < 0.05] at 95% confidence level)

P = Expected proportion of atherosclerosis in the coronary arteries based on a study done by Julius Ogeng'o et al which was 14.8% (37).

d = Confidence interval of 7 %

- Substitution into the formula **N = 95**

3.7 Sampling Method

Consecutive sampling which included all the decedents admitted at both KNH and City Mortuaries during the study period who fitted the above selection criteria.

3.7.1 Procedure

Each decedent was assigned a unique study number after obtaining informed consent. Selection of close relatives or guardians who had detailed knowledge of the decedent was done to ensure high accuracy of information was given about the decedent. Biodata, decedent's relevant medical and social history, and relevant family medical history data was collected from the relatives or guardians using a data collection tool. Included data were age, gender, personal history of the decedent of diabetes mellitus and hypertension, alcohol use, smoking, and family history of cardiovascular diseases like heart attacks or strokes (see appendix II).

3.7.2 Sample Collection

Height and waist circumference of the decedents was first measured and recorded with the height taken from head to heel and waist circumference taken at the level of the iliac crest with consequent calculation of Waist Height Ratio (WHR) as a marker of obesity. Postmortems were done on the decedents to determine the cause of death. The cause of death was documented according to the postmortem report

for each case. The hearts were excised, weighed and consequently placed in 10% formalin to fix for 1 week. Thereafter the coronary arteries were identified; the right coronary artery and the left coronary arteries - the left main coronary artery then branching into the left circumflex artery and then the left anterior descending artery. Luminal dissection and excision was done at 5mm intervals to locate if there was presence of gross atherosclerotic plaques, thrombi, luminal narrowing or calcification. Samples were then taken of each artery and placed into properly labelled individual cassettes with minimum of five sections per cassette. They were consequently placed into 10% formalin and thereby taken to the UON Histopathology Laboratory for processing.

3.7.3 Specimen Processing

The cassettes containing the sections of the coronary arteries were placed in a Leica Automated Tissue Processor. After tissue processing and paraffin embedding, sections of 5 micrometer were cut by a rotary microtome. Staining of all the sections was done with both Haematoxylin and Eosin (H&E) and Masson's Trichrome (MT) following laid down standard operating procedures (SOPS) (see appendix IV).

3.7.4 Histological Assessment

All slides processed were assessed for the presence of atherosclerotic lesions. Atherosclerotic lesions were microscopically classified on a reporting proforma (Appendix I) according to the American Heart Association (AHA) classification (40).

Table 1: American Heart Association Classification of Atherosclerosis

Type of Atherosclerosis	Histological Features
Type 0	Normal intima with no lipid/Adaptive intimal thickening
Type 1	Single lipid laden macrophages
Type 2	Multiple foam cells but no extracellular lipid pools
Type 3	Extracellular lipid pools but no lipid core
Type 4	Extracellular lipid core(s) with normal intimal surface
Type 5	Extracellular lipid core(s) with either a reactive fibrous cap, vascularisation or calcification
Type 6	Type 5 lesions with surface defect, haemorrhage, ulceration or thrombus

Type 1 and 2 are considered non-progressive atherosclerotic lesions while Types 3 and 4 are progressive atherosclerotic lesions and considered as intermediate lesions. Types 5 and 6 have the greatest risk of complications and considered as advanced lesions.

3.8 Quality Assurance

Accurate identification, clear labeling of the cassettes and slides and matching with the decedents was done to avoid any botching of collected samples. Tissue processing and staining followed laid down SOPs and was done by an experienced histotechnologist. All the slides which had evidence of atherosclerosis were confirmed and graded by two pathologists.

3.9 Variables

Dependent variables included anthropometric measures, site of atherosclerosis and histological classification of atherosclerosis. Independent variables included age, gender, alcohol intake, smoking, hypertension, diabetes and family history of cardiovascular diseases.

3.10 Ethical Considerations

Approval was obtained from Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (KNH/UON ERC) before conducting the study.

Permission via a written informed consent was sought from the relatives or guardians of the deceased who were adequately informed about the nature and purpose of the study. They were informed that the tissue samples collected were to be used for research purpose only.

Confidentiality of the decedents' data was maintained with only the principle investigator, supervisors and statistician allowed to view the data. No monetary incentives were offered to the families of the deceased.

3.11 Data Analysis

All the data was entered in a structured form using the study data collection tool. Using the assigned study number, data was entered into MS Excel sheet and verified to ensure there were no input errors. The data was then grouped and imported into SPSS20.0 statistical software for analysis.

Data was stratified according to age, gender, risk factors, anthropometric measurement, site and the type of atherosclerotic changes. Results were presented using tables, pie charts and graphs and where indicated photomicrographs.

4.0 CHAPTER FOUR: RESULTS

4.1 General Characteristics of the Decedents

Out of the 99 decedents, 95 met the inclusion criteria and 4 were rejected due to the presence of autolysis. The characteristics of the decedents were as outlined. Males accounted for 83.2% (n=95) of the decedents. Majority of the decedents, 34.7% were aged 21- 25 years, closely followed (32.6%) by decedents aged 30-35 years. The age range was 18-35 years. Only 2.1% of the decedents were hypertensive. Majority of the decedents, 52.6% were current drinkers and 60% (n=95) were nonsmokers. Most of the decedents had normal bodyweight (80%, n=95) as shown in below (Table 1).

Table 2: General Characteristics of the decedents

Variable	All n (%)	Normal n (%)	Atherosclerosis n (%)
Sex			
Female	16 (16.8%)	7 (15.9%)	9 (17.6%)
Male	79 (83.2%)	37 (84.1%)	42 (82.4%)
Age group			
15-20y	5 (5.3%)	3 (6.8%)	2 (3.9%)
21-25y	33 (34.7%)	20 (45.5%)	13 (25.5%)
26-30y	26 (27.4%)	11 (25%)	15 (29.4%)
30-35y	31 (32.6%)	10 (22.7%)	21 (41.2%)
Hypertensive			
No	93 (97.9%)	44 (100%)	49 (96.1%)
Yes	2 (2.1%)	0 (0%)	2 (3.9%)
Alcohol History			
Current Drinker	50 (52.6%)	29 (65.9%)	21 (41.2%)
Non-Drinker	36 (37.9%)	13 (29.5%)	23 (45.1%)
Past Drinker	9 (9.5%)	(4.5%)	7 (13.7%)
Cigarette History			
Current Smoker	35 (36.8%)	21 (47.7%)	14 (27.5%)
Non-Smoker	57 (60%)	22 (50%)	35 (68.6%)
Past Smoker	3 (3.2%)	1 (2.3%)	2 (3.9%)
Waist Height Ratio			
Normal	76 (80%)	36 (81.8%)	40 (78.4%)
Overweight	19 (20%)	8 (18.2%)	11 (21.6%)

4.2 Age and Gender of the Decedents

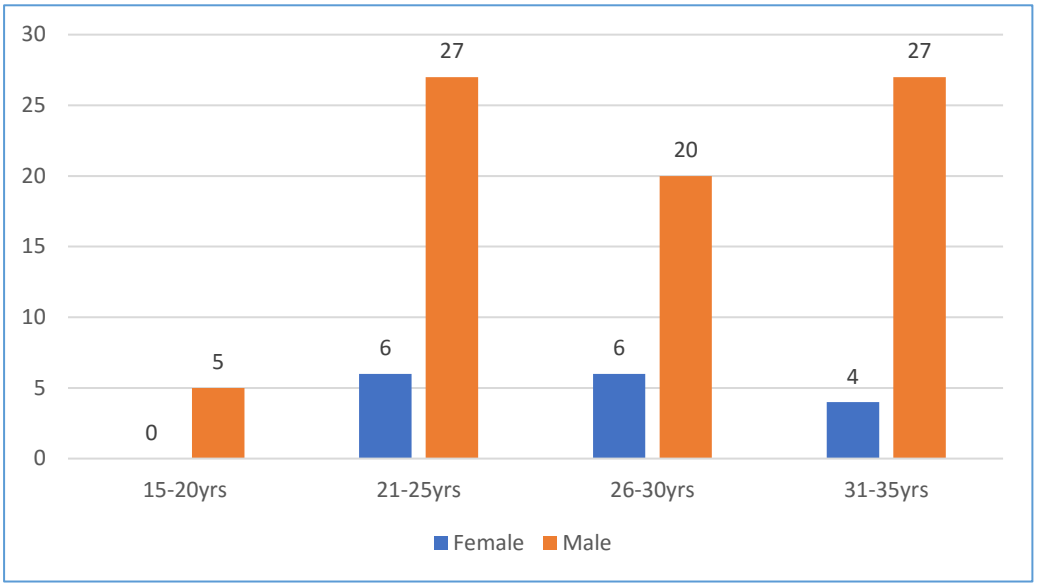

Figure 1: Age and gender of the 95 decedents at KNH & City Mortuaries, 2017

Figure 1 shows that the highest number of decedents were in the 21–25 year age bracket accounting for 34.7 % (n=95) of the total decedents while the lowest was in the 15–20 years age bracket accounting for 5.3 % (n=95) of the decedents. There were 31 decedents (32.6 %) in the 31–35 year age bracket and 26 decedents (27.5 %) in the 26–30 year age bracket. The mean age for both sexes was 27.55 years with mean age for men being 27.48 years and mean age for females being 27.88 years.4.3 Classification of causes of death by postmortem in adult African Kenyan youth between 18 and 35 years of age (n=95).

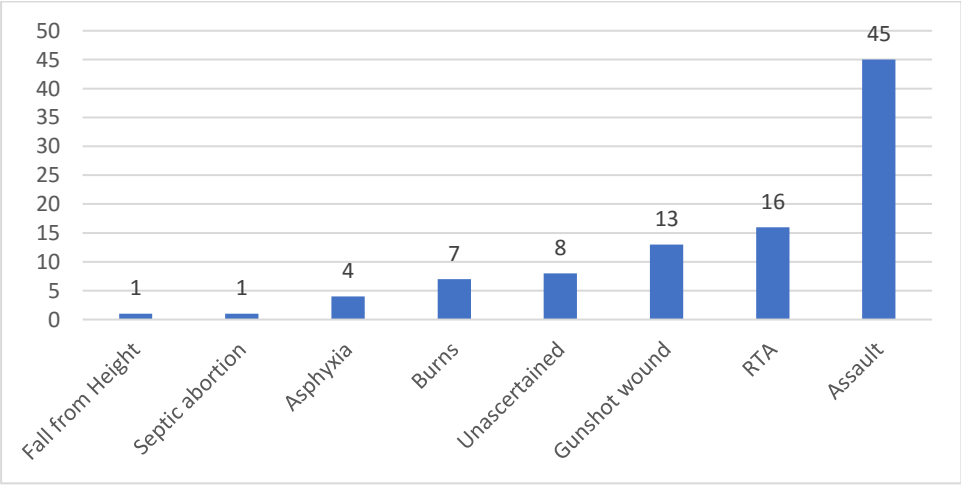

Figure 2: Classification of Causes of Death at Postmortem, 95 decedents at KNH & City Mortuaries, 2017

As shown in Figure 2 above, assault was the most common cause of death accounting for 47.4% (n=95), RTAs accounted for 16.8% (n=95) while gunshot injuries contributed to 13.7% (n=95). All other causes of death contributed to less than 10% (n=95) with 8.4% (n=95) of the cause of death unascertained.

4.4 Prevalence of Atherosclerosis

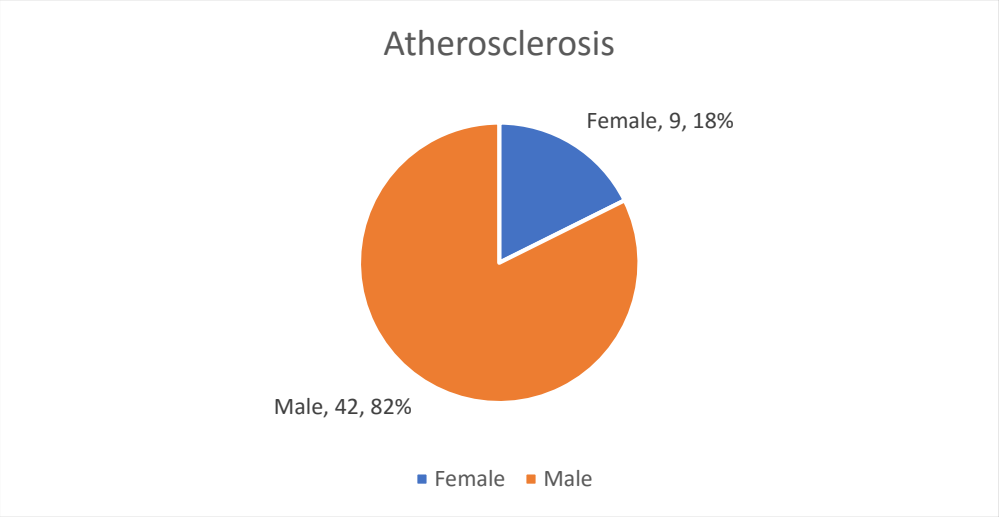

Figure 3: Proportion of the 95 decedents with Atherosclerosis on Postmortem, KNH & City Mortuaries, 2017

An identifiable atherosclerotic lesion in one or more of their coronary arteries was found in 51 (53.7 %) decedents. Out of this 42 (82%) were males as shown in figure 3 above.

4.5 Prevalence of Atherosclerosis according to the Number of Vessels

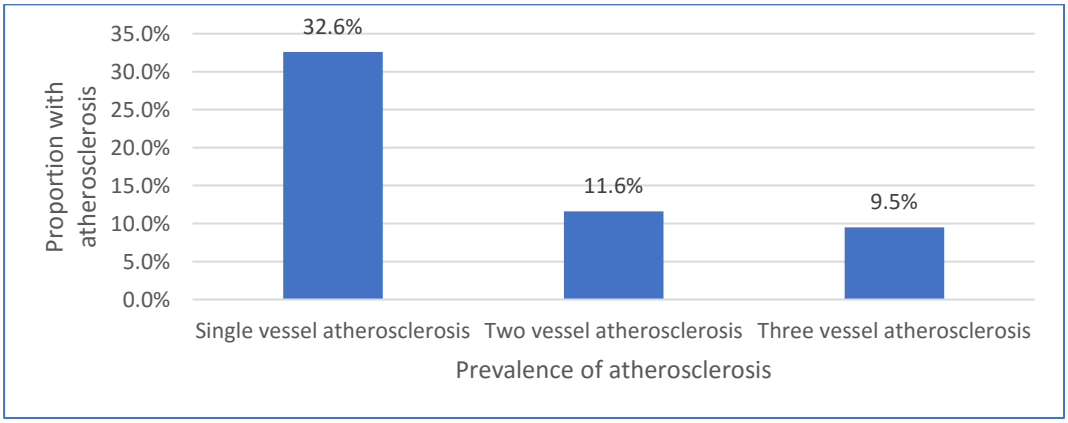

Figure 4: Prevalence of atherosclerosis according to the number of vessels in 95 decedents, KNH & City Mortuaries, 2017

Figure 4 showed that 31 (32.6%) decedents had single vessel atherosclerosis and that it was the most prevalent type of atherosclerosis. Two vessel atherosclerosis was identified in 11 (11.6%) of the decedents while three vessel atherosclerosis was identified in 9 (9.5%) of the decedents.

4.6AHA Classification according to age groups per coronary artery

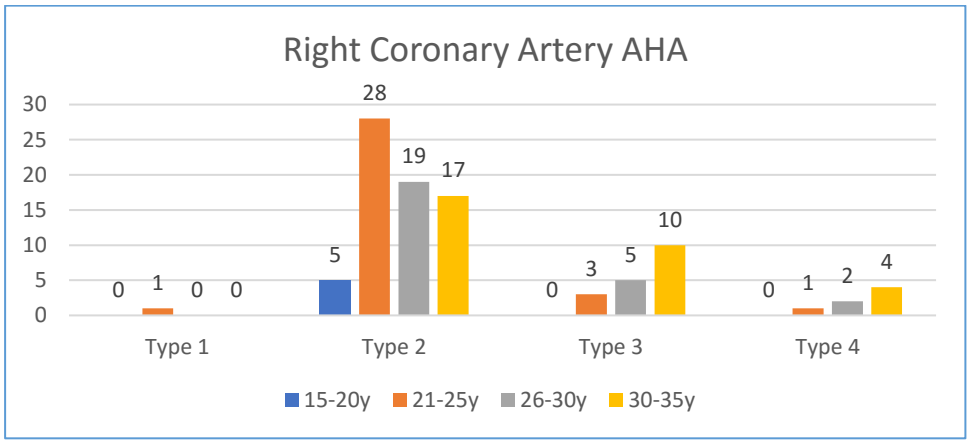

Figure 5: AHA Classification of Right Coronary Artery in different age groups of 95 decedents at KNH & City Mortuaries, 2017

Type 2 lesion was the most common atherosclerotic lesion in the Right Coronary Artery and most prevalent among decedents aged 21 – 25 years. There were more decedents aged 30-35 years with type 3 and type 4 lesions. No decedent had a type 5 lesion in the Right Coronary Artery (Figure 5).

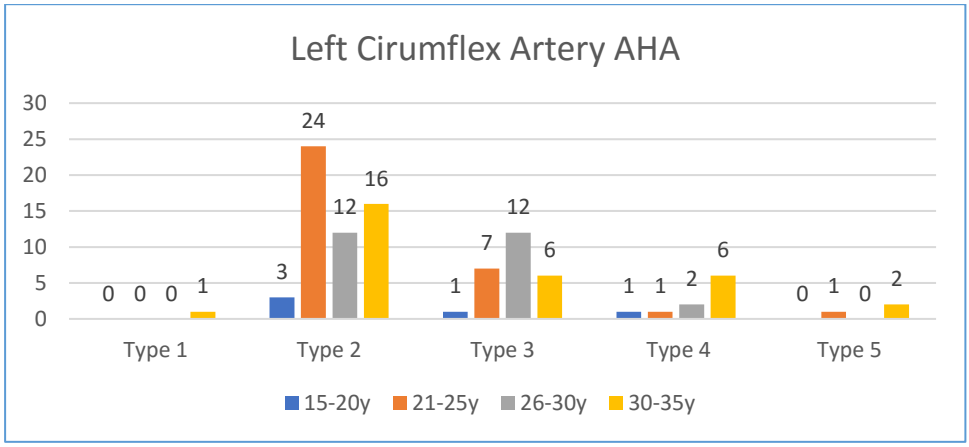

Figure 6: AHA Classification of Left Circumflex Artery in different age groups of 95 decedents at KNH & City Mortuaries, 2017

Type 2 lesion was the most common atherosclerotic lesion in the Left Circumflex Artery and the most prevalent among decedents aged 21 – 25 years followed by the age group 30 – 35 years. Three decedents had type 5 lesion in the Left Circumflex Artery (Figure 6).

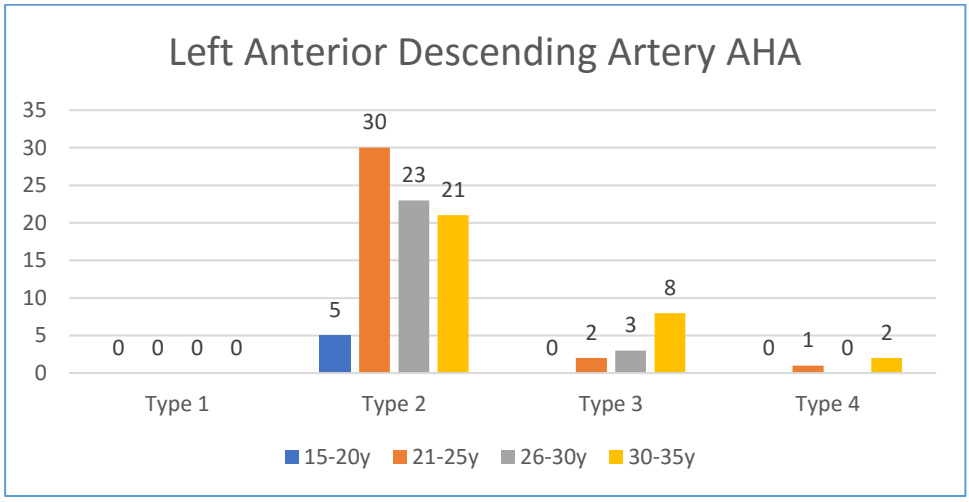

Figure 7: AHA classification of Left Anterior Descending Artery in different age groups of 95 decedents at KNH & City Mortuaries, 2017

Type 2 lesion was the most common atherosclerotic lesion in the left anterior descending artery. This was most prevalent among those aged between 21 and 25 years and was also common among those in the age group of 30 – 35 years. There was no decedent with type 5 atherosclerotic lesion (Figure 7).

4.7 Heart Weight

Table 3: Mean Heart Weights in grams

Descendants	n	Mean Heart Weight (gms)	Std. Dev.	Min	Max
All	95	277.5032	40.0	180.8	392.9
Normal	44	275.2068	37.9	197.4	373.1
Atherosclerosis	51	279.4843	42.0	180.8	392.9

The mean heart weight among all the decedents was 277.5 grams. The mean heart weight among those with no atherosclerotic lesions was 275.2 grams while among those with atherosclerotic lesions was 279.5 grams.

4.8 Types of Atherosclerosis (AHA) Per Coronary Artery

Twenty-five (26.3%) decedents had right coronary artery atherosclerosis, 39 (41.1%) had atherosclerosis in left circumflex artery and 16 (16.8%). The prevalence of each artery (RCA, LCA & LAD) atherosclerosis and the AHA type distribution was as shown in Table 3 below.

Table 4: Types of atherosclerosis (AHA) per coronary artery in 95 decedents at KNH & City Mortuaries, 2017

	AHA Type	**n (%)**
Right Coronary Artery	Type 1	1 (1.1%)
	Type 2	69 (72.6%)
	Type 3	18 (18.9%)
	Type 4	7 (7.4%)
Left Circumflex Artery	Type 1	1 (1.1%)
	Type 2	55 (57.9%)
	Type 3	26 (27.4%)
	Type 4	10 (10.5%)
	Type 5	3 (3.2%)
Left Anterior Descending Artery	Type 2	79 (83.2%)
	Type 3	13 (13.7%)
	Type 4	3 (3.2%)

Table 5: Logistic regression analysis of association of known risk factors with atherosclerosis in 95 decedents at KNH & City Mortuaries, 2017

Risk factors	Atherosclerosis n (%)	OR (95% CI)	P Value
Sex			
Female	9 (56%)	Reference	
Male	42 (53%)	0.9 (0.3 – 2.6)	0.821
Age group			
15-20y	2 (40%)	Reference	
21-25y	13 (39%)	0.98 (0.1 – 6.7)	0.979
26-30y	15 (58%)	2 (0.3 – 14.4)	0.472
30-35y	21 (68%)	3.2 (0.5 – 21.9)	0.247
Alcohol intake			
Current drinker	21 (42%)	Ref	
Not drinker	23 (63.9%	2.4 (1.0 – 5.9)	0.047*
Past drinker	7 (77.8%)	4.8 (0.9 – 25.6)	0.064
Hypertension			
No	49 (53%)	Reference	
Yes	2 (100%)	1450630471.1 (0 - ∝)	0.999
Cigarette History			
Current Smoker	14 (40%)	Reference	
Non Smoker	35 (61%)	2.4 (1.0 – 5.6)	0.048*
Past Smoker	2 (67%)	3 (0.2 – 36.3)	0.388
Waist Height Ratio (WHR)			
Normal	40 (53%)	Reference	
Overweight	11 (58%)	1.2 (0.4 – 3.4)	0.681
Heart weight (grams)	-	1.0 (0.9-1.0)	0.602

*significant at the 0.05 level

Simple logistic regression was done to assess the relationship between known risk factors and atherosclerosis. Non-drinkers of alcohol were 2.4 times more likely to have atherosclerosis than current drinkers of alcohol (OR = 2.4, 95% CI = 1.0-5.9, P=0.047). There was no significant difference between past drinkers and current drinkers of alcohol as it relates to atherosclerosis. Non-smokers of cigarettes were 2.4 times more likely to have atherosclerosis when compared to current smokers (OR = 2.4, 95% CI = 1.0-5.6, P=0.048). There was no significant difference between past smokers and current smokers as it relates to atherosclerosis. There was no significant relationship between the other risk factors and atherosclerosis (P>0.05) as shown in Table 4 above.

5.0 CHAPTER FIVE: DISCUSSION

There has been a steady increase of atherosclerosis in the younger generation which initially was deemed to be an affliction of developed countries (2). Currently developing countries carry the major burden of CVD mortalities but unfortunately there is no accompanying data to determine the root cause (2). In the present study, the overall prevalence of atherosclerosis with involvement of one or more coronary arteries in the decedents was found to be high. This result was similar to other studies but our prevalence was higher than an earlier study done by McNamara et al in young American soldiers (41). This, in comparison, is also significantly higher than a local study in Kenya by Ogeng'o et al, who was assessing the prevalence of atherosclerotic lesions in the tunica adventitia (37). However, this study focused on atherosclerosis on only one layer, the tunica adventitia. Overall, this strengthens the hypothesis that asymptomatic atherosclerosis is a common entity in the young age group.

This study found relative increase in severity of atherosclerosis with increasing age. This corroborated with published findings of the PDAY study group which was a multi-center autopsy based study looking at risk factors associated with atherosclerosis development in the youth (42). This could be attributed to the chronic progressive nature of atherosclerosis with age coupled with prolonged exposure to the various risk factors. There was presence of atherosclerosis in the decedents under 20 years supporting findings in other studies which support commencement of atherosclerosis in the second decade of life (43).

Males had a higher preponderance of atherosclerosis according to this study. This similar to a study done by Otaki et al who found out being male is the strongest predictor of developing atherosclerosis in young individuals (44). In a study done by M. Modelli in young victims of violent deaths, a majority of the cases of coronary atherosclerosis was in men (45). This could be attributed partially to the high number of deaths in men secondary to violent causes. Also, the higher engagement in risk factors like alcohol intake and smoking in this gender may play a key role in development and subsequent progression of atherosclerosis.

In our study, the youngest subject with the most advanced lesion was 25 years of age with type 5 lesion and who had a history of alcohol intake but no history of smoking nor was there obesity. No history of diabetes in the decedents or positivity of cardiovascular incidences in the family like MI or stroke was elicited from the next of kin of the decedents. Since atherosclerosis is known to be associated with the risk factors which were assessed during the present study, the difference in presence and extent of atherosclerosis between those with risk factors and those without risk factors was not statistically significant (P value > 0.05). Even though there was significant prevalence of atherosclerosis, none of the decedents in our study had obesity (WHR > 0.6) though 21.6% of the decedents with atherosclerosis were overweight (WHR 0.5 – 0.6): this could point to the multi-factorial nature of atherosclerosis. In contrast, Wenzhen et al found out that there is a positive association between WHR and asymptomatic atherosclerosis (46).

There was no statistically significant association between heart weight and atherosclerosis but it was noted in the study that hearts with atherosclerosis had a slightly higher mean heart weight. This could be attributed to the consequent increased workload on the heart followed by compensatory hypertrophy.

Histopathology revealed all the coronary arteries had some degree of atherosclerosis. Even the youngest age group in this study had intimal thickening and foam cells present which was also the same findings in the PDAY study group (43).Type 2 lesions were the most common for each coronary vessel. This was in tandem with a the study done by M. Modelli et al in Brazil (45). Similarly, Suraj et al also found Type 2 lesions most prevalent for LCA and LAD and Type 1 lesions most prevalent in the RCA and where most of the decedents in their study were in the 30 – 40 years age group (47). Early atherosclerotic lesions have been shown to occur in the first decade of life so above is not an unexpected finding (5). Single vessel atherosclerosis was the most prevalent at 32.6% (n=95) of the cases. The above points to a generalization that majority of individuals have early reversible atherosclerotic lesions but there is a factor/factors which triggers/trigger progress to more irreversible and advanced lesions.

Lesions with extracellular lipid deposits and lipid cores (Type 3 and 4) are considered as progressive lesions and on an irreversible path to severe atherosclerosis and consequently cardiovascular events like MI or CVA; they have been noted to start from the second to the third decade of life which corresponded to our study (40). Progressive atherosclerotic lesions were present on the LCA at 41.1 % followed by RCA at 26.3 %. Locally, Ogengo et al, who were evaluating all age groups, found LAD was the most common artery involved by atherosclerosis in contrast to our findings. This could suggest the possibility of the LCA being the most involved coronary artery during early years with a shift to the LAD in later years in our setting (7). A study done by Joseph et al analyzing coronary atherosclerosis in young trauma victims found LCA was the most involved artery of which we had a similar finding but in contrast to our study, LAD was the second most involved (48).

The most advanced lesion seen was type 5 seen in 5.9% of those with atherosclerosis and the youngest decedent affected was 25 years old with history of alcohol intake being the only present risk factor assessed: all the cases of type 5 were seen only on the LCA and in males. This finding was unusual since type 5 lesions are typically encountered from the fourth decade of life and may point towards other modulating factors which hasten the development of atherosclerosis (40).

No surface defect, haemorrhage, thrombus or calcification (type 6 lesions) was noted in all the coronary arteries analysed in our age group which was similar to a study done by Rodríguez-Saldaña et al in Mexico City which showed severe lesions in more advanced age (49). This confirms the chronicity of atherosclerosis with end stage disease typically seen in much older age groups which is associated with higher morbidity and mortality.

5.1 Conclusions

There is presence of coronary atherosclerotic lesions in young decedents in our setting with a high prevalence noted. AHA Type 2 is the most frequent atherosclerotic lesion and the left circumflex artery is the most afflicted coronary artery with higher stages of atherosclerosis commonly seen among older age

groups. There was no significant relationship determined between the risk factors assessed and coronary atherosclerosis.

5.2 Limitation

Access to detailed medical data of the decedents was not possible in this study which would have aided substantially in determining the presence of any other underlying confounding variables that can affect atherosclerosis e.g. HIV.

5.3 Recommendations

Future studies that target a wider study population that incorporate epidemiologic data, detailed medical records and cardiovascular biomarkers could be done to further aid in analysis of atherosclerosis in the young adults in our setting.

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APPENDICES

Appendix I: Postmortem Information and Consent Form

POSTMORTEM INFORMATION AND CONSENT FORM:

This informed consent form has two parts:

1. Information sheet (to share information about the study)
2. Certificate of consent

PART 1: INFORMATION SHEET

Introduction

My name is DR KAMOTHO WATENGA. I am a postgraduate student in the Department of Human Pathology at the University of Nairobi. I am conducting this study to determine if there is presence of fatty deposits in the heart vessels of young people who have died and to determine if there any risk factors associated with this.

You as the next of kin of the deceased are invited to participate in this study. In case you do not understand any words used in this information sheet and have any questions, please ask me to stop and explain.

Type of Research Intervention

This study involves the analysis of arteries of the heart in young people admitted here in Kenyatta National Hospital mortuary. We will take tissue samples of these arteries which will then be sent to the laboratory for processing and then assessed under a microscope.

Participant Selection

We aim to recruit all the deceased admitted in Kenyatta National Hospital mortuary aged between 15 and 35 years of age.

Voluntary Participation

Your participation in this research is entirely voluntary and it is your choice whether to participate or not. Whether you choose to participate or not, all the services you and the deceased receive will not change.

Procedures and Protocol

Next of kin of eligible deceased participants will be asked to join the study. All will then be requested to sign a consent form. You will then be asked some questions which will be recorded in a questionnaire. Thereafter, a post-mortem will be conducted. During the post-mortem, the arteries of the heart will be sampled. This will then be taken to the laboratory to be processed then checked for presence of atherosclerosis (fatty deposits).

Harmful Effects

There are no harmful effects in this study.

Risks

There will be no risks expected with this study.

Benefits

We will be able to diagnose whether or not your deceased relative had fatty deposits in the heart vessels. Your participation will also help in the overall management of young adults with atherosclerosis.

Reimbursements

You will not be given any money or gifts to take part in this research.

Confidentiality

All participants will be identified using a number (names will not be used). All information shared by you during this study will be viewed by the researchers only.

Sharing the Results

The results obtained during this study will be shared with you. We will publish the results in order that other interested people may learn from it. However your identity or the identity of the deceased will never be revealed.

Request to Participate in the Study

Kindly indicate whether you are interested in joining this study. If you are willing to join the study I kindly request you to fill the consent certificate provided.

Right to Refuse

Should you decline to participate in this study, this will not affect services offered to you or the deceased in any way. You will still have all the benefits that you would have had otherwise.

Who to Contact

If you have any questions regarding this study at any time you may contact the principal investigator: DR. KAMOTHO WATENGA (0724669929) or any of my supervisors below:

Supervisors

1. **Dr. Wairimu Waweru:** P.O. BOX 19676-00202, Nairobi. Tel +254 722759523
2. **Dr. Edwin Walong:** P.O. BOX 19676-00202, Nairobi. Tel +254 738 590623
3. **Dr. Kuria Gikonyo:** P.O. BOX 19676-00202, Nairobi. Tel +254 721570812

You can also contact the Ethics and Research Committee at Kenyatta National Hospital (KNH/UON-ERC): P.O. BOX 20723-00202, Nairobi. Telephone +254 020 726300-9

PART II: CERTIFICATE OF CONSENT

I have read the foregoing information, or it has been read to me. I have the opportunity to raise any questions about my participation and that of the deceased in the study, and any questions I asked have been answered to my satisfaction. My rights have been explained to me and I consent voluntarily to participate in this study.

Print Name of Participant:

Relation to the Deceased:

Signature of Participant:

Date:

If Illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumbprint as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of Witness:

Signature of Witness:

Date:

Thumb Print of Participant:

FOMU YA IDHINI KICHWA CHA UTAFITI

UCHAMBUZI KATIKA UPASUAJI YA UGONJWA WA ATHEROSCLEROSIS AMBAYO INADHURU MISHIPA YA MOYO KATIKA UJANA WALIOKUFA KWA SABABU ISIYO YA ASILI

Jina langu ni DR KAMOTHO WATENGA mwanafunzi wa chuo kikuu cha Nairobi idara ya Human Pathology. Ningependa kufanya utafiti ambayo nitawaelezea. Tafadhali soma ujumbe ifuatayo kwa makini. Ujumbe huu itaelezwa kwa njia ya Kiingereza na Kiswahili. Una uhuru wa kuchagua lugha ambayo utaelewa vyema.

Maelezo kwa Ufupi:

Uchambuzi katika upasuaji ya ugonjwa wa atherosclerosis ambayo inadhuru mishipa ya moyo katika ujana waliokufa kwa sababu isiyo ya asili. Tutachukuasampuliyamishipakutokaaliyekufanakuipelekakwamaabarayachuokikuu cha Nairobi. Huko tutapima kutambua kama kuna ugonjwa wa atherosclerosis katika mishipa.

Faida na Tatizo ya Utafiti Huu:

Mishipa yatayopatikana kutoka aliyekufa itachunguzwa ugonjwa wa atherosclerosis. Utanufaika kwa kupata matokeo ya sampuli kuhusu ugonjwa wa atherosclerosis. Hakuna madharaa yoyote kutoka utafiti huu.

Taratibu wa Kushiriki:

Watakaoshiriki katika uchunguzi huu itakuwa kwa njia la hiari bila kushuritishwa. Ukiamua kutoshiriki, hautapoteza kwa njia yeyote haki yako au ya marehemu ya kuhudumiwa unavyostahili. Majibu ya uchunguzi huu utapewa.

Idhini ya Mshiriki:

Watakaoshiriki katika utafiti huu itakuwa kwa hiari bila kusurutisha. Una uhuru wa kutoshiriki, kutojibu swali lolote kwenye dodoso au kukatiza kipindi cha maswali iwapo hautaridhika na jambo lolote.

Pia waweza kutamatisha ushirika wako kwenye utafiti huu bila kupoteza haki yako ya kushughulikiwa katikachumba cha kuhifadhia maiti hii.

Anwani:

Mchunguzi, DR KAMOTHO WATENGA, Chuo Kikuu Cha Nairobi SLP 19676-00202 Nairobi Nambari ya simu 0724669929. Pia unaweza kutafuta wasimamizi wafuatayo:

Wasimamizi:

1. Dr Wairimu Waweru: S.L.P 19676-00202, Nairobi. Nambari ya Simu +254 722759523
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4. KNH/UON-ERC S.L.P 20723-00202, Nairobi. Nambari ya Simu +254 020 726300-9

Idhini ya Mshiriki:

Kama utashiriki tafadhali tia sahihi yako kwenye pengo lilioachwa hapa chini.

Mimi……..….. nimesoma na nimeelewa nia ya uchunguzi huu, utaratibu utaotumika kuchukua kipimo, faida na madhara yanayohusika

na uchunguzi huu. Nimekubali kushiriki kwa hiari bila kushurutishwa.

Sahihi ya Mshiriki……. Tarehe…….

Sahihi ya Shahidi…….. Tarehe……..

Statement of the researcher/ person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands what the research is all about.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability.

I confirm that the individual has not been coerced into giving consent and the consent has been given freely and voluntarily.

A copy of this document has been provided to the participant.

Print Name of Researcher/person taking the consent:……

Signature of Researcher/person taking the consent:……

Date:……

Appendix II: Study Data Collection Form

Autopsy No. ----------Study No.: ----------

Name of Next Of Kin, Relation To Deceased And Contact: ----------

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Part A

1 – Patient Details

Date of Post Mortem: ----------Age (Yrs): ----------

Gender: Male □ Female □

Cause of Death: ----------

2 - Clinical History

a. Personal History of:

i. Hypertension Yes □ No □ Unknown □

ii. Diabetes Mellitus Yes □ No □ Unknown □

b. Family History of CVDs Yes □ No □ Unknown □

If present, specify ----------

c. Social History

Alcohol Consumption: Current Drinker □ Past Drinker □ Never Drinker □

Cigarette Smoking: Current Smoker □ Past Smoker □ Never Smoker □

Swahili

Historia Ya Kiafya

d. Historia Kibinafsiya:

i. Ugonjwa wa Mishipa: Ndio □ La □ Haijulikani □

ii. Ugonjwa wa Sukari: Ndio □ La □ Haijulikani □

e. Historia kwa Familia ya Ugonjwa za Moyo

Ndio □ La □ Haijulikani □

Kama liwelo, fafanua----------

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f. Historia Ya Kijamii

Matumizi Ya Pombe

Mnywaji Hivi Sasa □ Mnywaji wa Kitambo □ Mto Mnywaji □

Mvutaji Sigara

Mvutaji Hivi Sasa □ Mvutaji wa Kitambo □ Mto Mvutaji □

Part B

Section 1: Gross Features

General Examination

a. Height (Cm) ----------

b. Waist Circumference(Cm) ----------

c. WHR……...

1. Heart Weight (Gm) ----------

Other Notable Gross Features----------

Section 2: Coronary Arteries

Artery	Gross Features	Histological Features	Histological Grading
Right Coronary Artery			
Left Circumflex Artery			
Left Anterior Descending Artery			

Gross Features: Thrombus, Stenosis, Atherosclerotic Plaque, Calcification

Histologic Features: Macrophages, Foam Cells, Intimal Thickening, Lipid Deposition, Fibrosis, Haemorrhage, Thrombosis, Ulceration, Necrosis, Calcification

Appendix III: AHA Histological Classification of Atherosclerosis

a. Type I lesion

Increase in polymorphonuclear cells and monocytes in the sub endothelial space increase in macrophages and formation of scattered macrophage foam cells.

b. Type II lesion

Consist primarily of layers of macrophage foam cells and lipid-laden smooth muscle cells and include lesions grossly designated as fatty streaks.

c. Type III lesion

In addition to the lipid-laden cells of type II, type III lesions contain scattered collections of extracellular lipid droplets and particles that disrupt the coherence of some intimal smooth muscle cells.

d. Type IV lesion

Also called atheroma. There is presence of lipid core in the extracellular space.

e. Type V lesion

Type Va - Presence of lipid core and in addition smooth muscle cells and fibroblast (fibroatheromas)

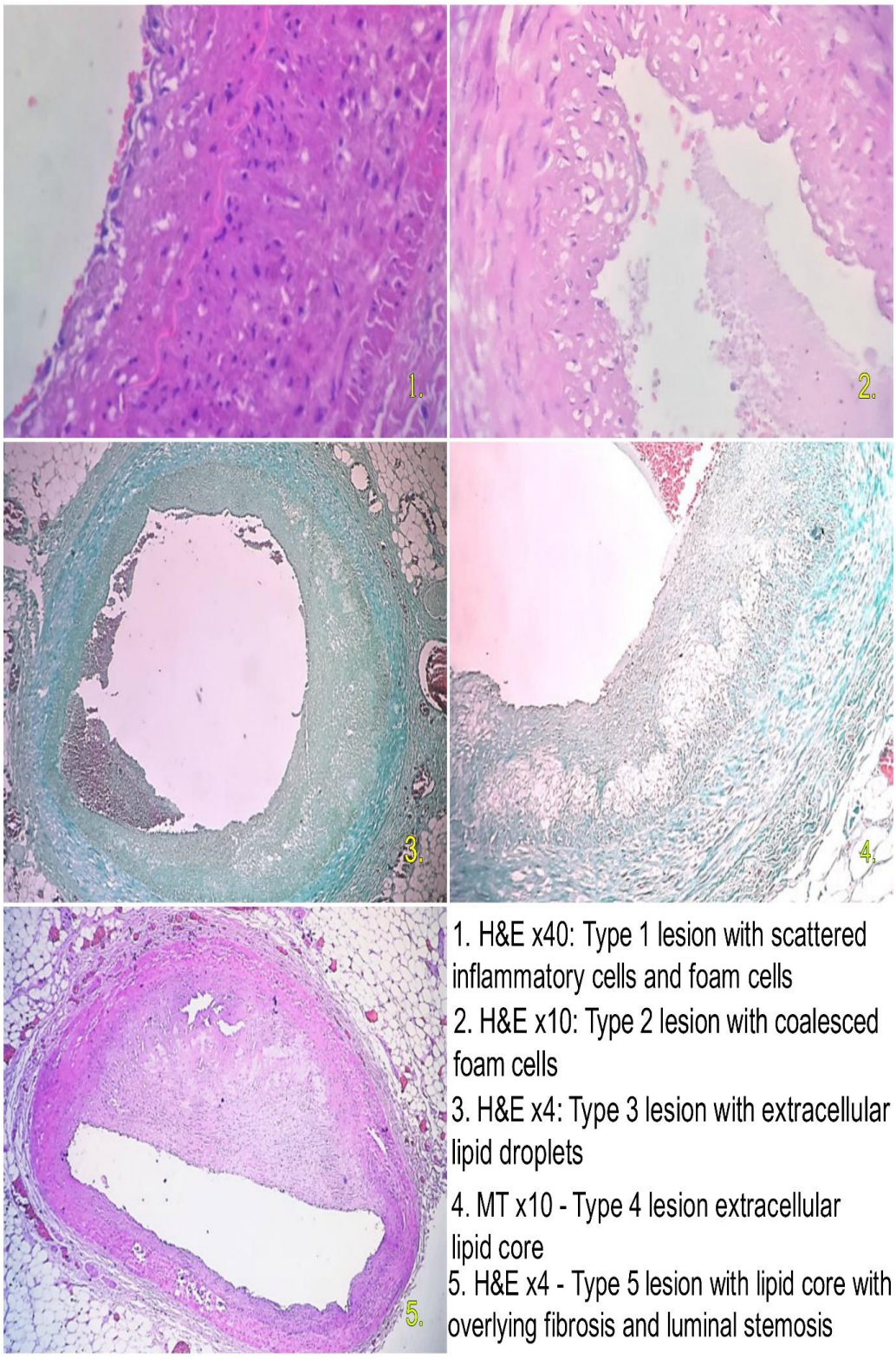
Type Vb – Calcified lesion

Type Vc – Fibrotic lesion with little or no accumulated lipid or calcium

f. Type VI lesion

Type V lesion and/or presence of fissure, haematoma or thrombus

Appendix IV: Photomicrographs of Atherosclerotic Lesions



1. H&E x40: Type 1 lesion with scattered inflammatory cells and foam cells
2. H&E x10: Type 2 lesion with coalesced foam cells
3. H&E x4: Type 3 lesion with extracellular lipid droplets
4. MT x10 - Type 4 lesion extracellular lipid core
5. H&E x4 - Type 5 lesion with lipid core with overlying fibrosis and luminal stemosis

Appendix V: SOPS for Tissue Staining

A. HAEMATOXYLIN AND EOSIN STAINING

PURPOSE: Haematoxylin and Eosin (H&E) staining is the most common staining technique in histopathology. This uses a combination of two dyes, Haematoxylin and Eosin used for demonstration of nucleus and cytoplasmic inclusions in clinical specimens.

PRINCIPLE: Alum acts as mordant and hematoxylin containing alum stains the nucleus light blue. This turns red in presence of acid, as differentiation is achieved by treating the tissue with acid solution. Bluing step converts the initial soluble red color within the nucleus to an insoluble blue color. The counterstaining is done by using eosin which imparts pink color to the cytoplasm.

CONTROL: Any well stained tissue

FIXATIVE: Any well-fixed tissue.

TECHNIQUE: Cut paraffin sections 4 to 5 μm

REAGENTS

1. Harris Haematoxylin stain

A = 1 gm haematoxylin in 10 ml ethanol

B = 20 gm ammonium alum in hot distilled water

Mix A & B, boil and add 0.5 gm of mercuric oxide and filter.

2. Eosin solution

Yellow eosin = 1 gm

Distilled water = 80 ml

Ethanol = 320 ml

Glacial Acetic Acid = 2 drops

3. 0.5% HCl

4. Dilute ammonia water

PROCEDURE

i. Deparaffinize the section: flame the slide on burner and place in the xylene. Repeat the treatment.
ii. Hydration: Hydrate the tissue section by passing through decreasing concentration of alcohol baths and water. (100%, 90%, 80%, 70%)
iii. Stain in haematoxylin for 3-5 minutes
iv. Wash in running tap water until sections "blue" for 5 minutes or less.
v. Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for 5 minutes.
vi. Wash in running tap water until the sections are again blue by dipping in an alkaline solution (e.g. ammonia water) followed by tap water wash.
vii. Stain in 1% Eosin Y for 10 minutes
viii. Wash in tap water for 1-5 minutes
ix. Dehydrate in increasing concentration of alcohols and clear in xylene
x. Mount in DPX
xi. Observe under microscope

RESULTS

- Nuclei Blue, black
- Cytoplasm Pink
- Muscle fibers Deep red
- RBCs Orange red
- Fibrin Deep pink

B. MASSON'S TRICHROME STAIN

PURPOSE: This stain is used to differentiate between collagen and smooth muscle in tumours, and the increase of collagen in diseases such as advanced atherosclerosis and cirrhosis. It is a routine stain for liver and kidney biopsies.

PRINCIPLE: Three dyes are employed selectively to stain muscle, collagen fibers, fibrin, and erythrocytes. The general rule in trichrome staining is that the less porous tissues are colored by the smallest dye molecule; whenever a dye of large molecular size is able to penetrate, it will always do so at the expense of the smaller molecule. Others suggest that the tissue is stained first with the acid dye, Biebrich Scarlet, which binds with the acidophilic tissue components. Then when treated with the phospho acids, the less permeable components retain the red, while the red is pulled out of the collagen. At the same time causing a link with the collagen to bind with the aniline blue.

CONTROL: Skin or lung.

FIXATIVE: Any well-fixed tissue.

TECHNIQUE: Cut paraffin sections 4 to 5 μm

REAGENTS:

1. **Bouin's Solution:**

Picric acid (saturated) = 75 ml

Formaldehyde (37-40%) = 25 ml

Glacial acetic acid = 5 ml

2. **Weigert's Iron Haematoxylin Solution:**

Stock Solution A:

Haematoxylin = 1 gm

95% Alcohol = 100 ml

Stock Solution B:

29% Ferric chloride in water = 4 ml

Distilled water = 95 ml

HCL, concentrated = 1ml

Mix equal parts of stock solution A and B. This working solution is stable for 3 months.

3. **Biebrich Scarlet-Acid Fuchsin Solution:**

Biebrich scarlet, 1% aqueous = 90 ml

Acid fuchsin, 1% aqueous = 10 ml

Acetic acid, glacial = 1 ml

4. **Phosphomolybdic-Phosphotungstic Acid Solution:**

5% Phosphomolybdic acid = 25 ml

5% Phosphotungstic acid = 25 ml

5. **Light Green Solution:**

Light green = 5gm

Distilled water = 250 ml

Acetic acid, glacial = 2 ml

6. **1% Acetic Acid Solution:**

Acetic acid, glacial = 1 ml

Distilled water = 99 ml

PROCEDURE

i. Deparaffinize and rehydrate through 100% alcohol, 95% alcohol 70% alcohol.
ii. Wash in distilled water. Rinse running tap water for 5-10 minutes to remove the yellow color.
iii. Stain in Weigert's iron hematoxylin working solution for 10 minutes.
iv. Rinse in running warm tap water for 10 minutes.
v. Wash in distilled water.
vi. Stain in Biebrich scarlet-acid fuchsin solution for 10-15 minutes. Solution can be saved for future use.
vii. Wash in distilled water.
viii. Differentiate in phosphomolybdic-phosphotungstic acid solution for 10-15 minutes or until collagen is not red.
ix. Transfer sections directly (without rinse) to light green solution and stain for 5-10 minutes. Rinse briefly in distilled water and differentiate in 1% acetic acid solution for 2-5 minutes.
x. Wash in distilled water.

xi. Dehydrate very quickly through 95% ethyl alcohol, absolute ethyl alcohol (this step will wipe off Biebrich scarlet-acid fuchsin staining) and clear in xylene.

xii. Mount in DPX.

xiii. Observe under microscope

RESULTS:

- Collagen Green
- Nuclei Black
- Muscle, Cytoplasm, Keratin Red

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Appendix VI: KNH/UON Ethical Approval

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KENYATTA NATIONAL HOSPITAL APPROVED 09 MAR 2017 KNH/UoN-ERC P.O. Box 20723-00202 Nbi

Ref: KNH-ERC/A/76

8th March 2017

Dr. Michael Kamotho Watenga
Reg.No.H58/74641/2014
Dept.of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr.Watenga

REVISED RESEARCH PROPOSAL: POSTMORTEM ANALYSIS OF CORONARY ATHEROSCLEROSIS AMONG YOUTH DYING OF UNNATURAL CAUSES (P640/09/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 8th March 2017 – 7th March 2018.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

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