HISTOMORPHOLOGICAL FEATURES OF ATHEROSCLEROSIS IN THE LEFT ANTERIOR DESCENDING CORONARY ARTERIES AMONG BLACK KENYANS

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ABSTRACT

The pattern of coronary artery atherosclerosis is valuable in informing mitigation strategies for coronary heart disease. Histomorphological data on this disease among Africans living in Sub Saharan Africa are, however, scarce. The left anterior descending is one of the most commonly afflicted arteries. This study, therefore, examined the left anterior descending artery of 213 black Kenyans [Mean age 36.8 years, range 5 – 82 years] who had died of non cardiovascular causes for features of atherosclerosis. The individuals were divided into male and female, then into 10-yr age groups. Specimens were obtained from the proximal segment of the artery during autopsy at the Department of Human Anatomy University of Nairobi, Kenya. They were processed routinely for paraffin embedding and sectioning. Five micron sections were stained with Haematoxylin/Eosin and Mason's trichrome and examined with light microscope. Micrographs of representative features were taken using a high resolution digital camera. At least one feature of atherosclerosis was present in 54 (25.4%) of the individuals. The features observed included severe intimal hyperplasia (34; 63%), disintegration of the internal elastic lamina [30; 55.6%]; atherosclerotic plaque (20; 37%), adventitial thicknening (14; 26%) and mural neovascularization (10; 18.5%). The mean age of those with features of atherosclerosis was 38.4 years, range 6 – 62 years with 25 (46.3%) being aged 40 years and below. Of these, the male: female ratio was 1.7:1. In conclusion, features of atherosclerosis are present in over 25% of the population studied. The disease affects young people, including women. Proactive preventive measures including follow – up should commence early, and involve both men and women

Keywords: Atherosclerosis, coronary, young, men, women, Kenya

INTRODUCTION

Atherosclerosis of coronary arteries is one of the most common causes of ischemic heart disease (IHD) [Marzilli et al., 2012; Jha et al., 2013]. The incidence of IHD is rising in Sub Saharan Africa (SSA) consequent to epidemiological and demographic transitions (Mensah, 2008; Nkoke and Luchuo, 2015). Currently, the disease constitutes a substantial cause of morbidity and mortality in the region (Ikem and Sumpio, 2011; Essien et al., 2014; Ebireri et al., 2016). In Kenya, acute myocardial infarction is not uncommon (Ogeng'o et al., 2010a), is the most common cause of cardiovascular deaths (Ogeng'o et al., 2011) and may cause 10 – 20% of heart failure (Ogeng'o et al., 2014a; Bloomfield et al., 2016). There is fear of a looming epidemic of coronary heart disease (CHD) unless deliberate measures are taken to mitigate the imminent rise (Churchill, 2013; Ebireri, 2016). Reliable data are important to inform these measures. Histopathological studies on coronary artery

disease (CAD) are an important source of data for improving understanding of the disease (Abedinzadeh et al., 2015) but are scarce from SSA. Among Kenyans, we recently demonstrated features of atherosclerosis in over 20% of carotid arteries (Ogeng'o et al., 2017) but studies on coronary arteries are scarce. The left anterior descending (LAD) artery is one of the most commonly afflicted arteries (Golshahi et al., 2005; Vyas et al., 2015). This study therefore investigated the distribution of histomorphological features of atherosclerosis in proximal LAD among black Kenyans.

MATERIALS AND METHODS

This study was done on autopsy material from samples of 213 victims of trauma at the Department of Human Anatomy, University of Nairobi for whom the next of kin were able to provide adequate medical history. It included only individuals whose next of kin reported no record of cardiovascular disease or history of the conventional risk factors namely hypertension, diabetes mellitus, cigarette smoking, atherogenic diet, overt psychosocial stress, HIV infection or antiretroviral therapy. Ethical approval was provided by the Kenyatta National Hospital / University of Nairobi Ethics and Research Committee. Informed consent was provided by the next of kin who brought the body to the Chiromo Funeral Parlour. Permission was granted by the Chairman of Department. The cases were obtained by systematic sampling in which every 4th case from individuals who had died of trauma were examined. The age range was 6 – 82 years. The subjects were divided into male and female and then into 10 year age groups as follows: 0 – 10, 11 – 20, 21 – 30, 31 – 40, 41 – 50, 51 – 60, 61 – 70, 71 – 80 and over 80 years. The pericardium was removed, the left coronary artery (LCA) and its LAD branch identified. Cases of variant branching of LCA were excluded from the study, to eliminate the concomitant anatomical predisposition to atherosclerosis.

Two (2) mm long specimens were taken from the proximal LAD within 72 hours of death, to avoid overt postmortem damage to the tissues. They were fixed by immersion in 10% formaldehyde solution for three days, then trimmed and routinely processed for paraffin embedding by passing them through increasing concentrations of isopropyl alcohol, and cleared with cedar wood oil for 12 hours. The sections were then infiltrated with fresh molten paraplast wax for 12 hours. Seven micrometer sections were stained with Haematoxylin/Eosin and Mason's trichrome for demonstration of the general organization of the mural components. The slides were then examined at various magnifications for conventional features of atherosclerosis namely intimal hyperplasia, degeneration of the tunica media, atherosclerotic plaques, adventitial thickening and neovascularization. Micrographs of representative features were taken using a high resolution digital camera (Sony – DSC – HX400 20.4 – Megapixel ©) attached to a photomicroscope.

RESULTS

Of the 213 cases, there were 135 males and 78 females, distributed in 9 ten - year age groups. Mean age was 36.8 years (range 5 – 82 years). One hundred and thirty nine of them (65.2%) were aged below 50 years with 23 (10.8%) being under 20 years. Twelve (5.6%) of them were in the first decade. One hundred and sixty three (76.5%) were aged 21 – 50 and 115 (54%) were between 21 – 40 years (Fig 1).
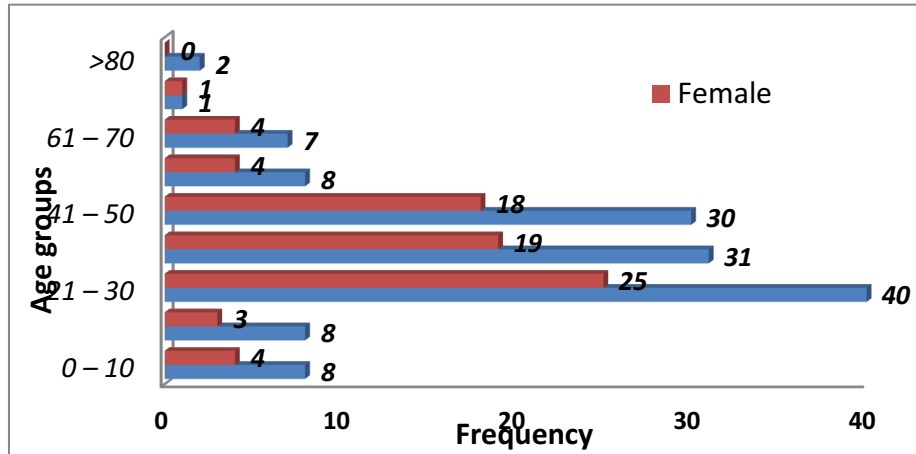
Figure 1: Age Distribution of the population whose LAD was studied

General Observations

The LAD was a muscular artery with the conventional three tunics namely tunica intima, media and adventitia (Fig 2a,b). At least one feature of atherosclerosis was present in 54 (25.4%) of all cases. In total, 6 main features of atherosclerosis were observed, namely severe intimal hyperplasia, atherosclerotic plaque, disintegration of IEL, medial degeneration, adventitial thickening and mural neovascularization (Table 1). In 34 (63%), cases there was severe intimal hyperplasia (Fig 2c). In 17 (50%) of these cases, the tunica intima alone was greater than the tunica media and adventitia combined (Figure 2d) and in 11 (32.4%) of the cases it was so severe that it occluded the lumen (Figure 2e). In 20 (37%) cases, parts of the intima had degenerated due to atherosclerotic plaque formation (Figure 2f). The tunica adventitia was remarkably thickened in the absence of intimal hyperplasia in 14 (25.9%) cases [Fig 2a]. Neovascularization of the tunica adventitia was evident (Fig 2c) in 10 (18.5%) of cases (Table 1).

Table 1: Distribution of features of atherosclerosis

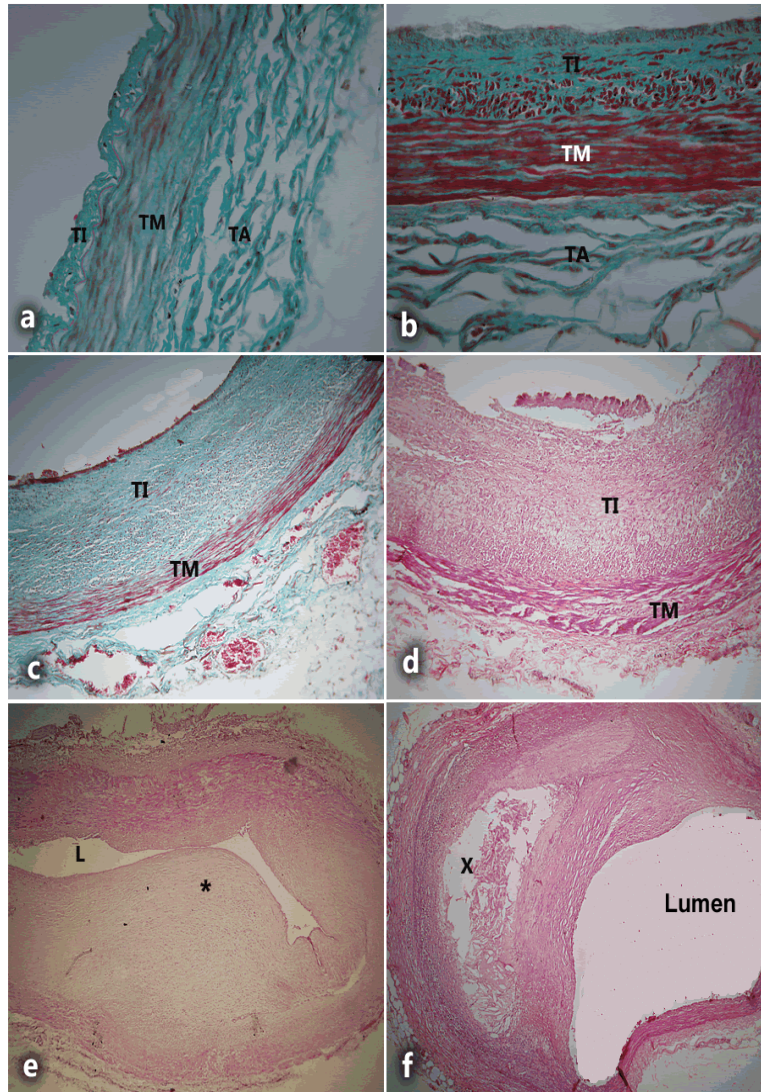| Feature	Frequency			(%)
	M	F	Total	
Severe Intimal Hyperplasia	22	12	34	63
Disintegration of IEL	18	12	30	55.6
Atherosclerotic Plaque	12	8	20	37
Medial degeneration	10	10	20	37
Adventitial thickening alone	8	6	14	25.9
Mural neovascularization	5	4	10	18.5

Fig 2 a - f: Degrees of intimal hyperplasia in proximal LAD of black Kenyans. TI = Tunica intima; TM = Tunica media; TA = Tunica adventitia. Mason's trichome and Hematoxylin/Eosin x100. A. Full wall thickness in LAD of 25 year old male showing the usual three tunics. B - Slight intimal hyperplasia in a 31 year old female. Note the tunica intima (TI) is about the same size as the tunica media (TM). C - Moderate to severe hyperplasia in a 36 year old male. Note the tunica intima (TI) is double the size of the tunica media (TM). D - Severe intimal hyperplasia in a 38 year old male. The tunica intima (TI) is more than double the tunica media (TM). E - Very severe intimal hyperplasia (star) in a 9 year old male. Note partial occlusion of the lumen, leaving only a slit lumen (L). F - Atherosclerotic plaque (x) in a 39 year old male.

The hypertrophied tunica intima was highly cellular. The cells were mainly observed in the deeper layer of intimal thickening and were of various shapes and sizes (Figure 2b - d; Fig 3a). They also took different

Degeneration of internal elastic lamina

The internal elastic lamina was often disintegrated at multiple sites to leave gaps.

orientations. One of the major limitations of this study was that the cells were not characterized. However, the different shapes of the nuclei suggest that they were of different varieties.

In some of the gaps, cells could be seen traversing between the tunica media and intima (Figure 3b). In cases of moderate to

severe intimal hyperplasia, the IEL was altogether not discernible and the tunica intima appeared to merge with the tunica media imperceptibly (Fig 2 c - e).

Degeneration of tunica media

The tunica media was, in many the cases, markedly attenuated (Figure 3c). As the tunica intima increased, the tunica media diminished in size. There were gaps between SMC, and spaces between the layers of smooth muscle, suggestive of degeneration

(Fig 3c, d). Incase of atherosclerotic plaque, the tunica media was not discernible.

Adventitial thickening

There was distinct thickening of the tunica adventitia in 14 (25.9%) of the cases. In several of them, the tunica adventitia was thickened with only slight intimal thickening (Figure 3d). In a few cases, the adventitial thickening preceded intimal thickening (Figure 2a; and 3d).
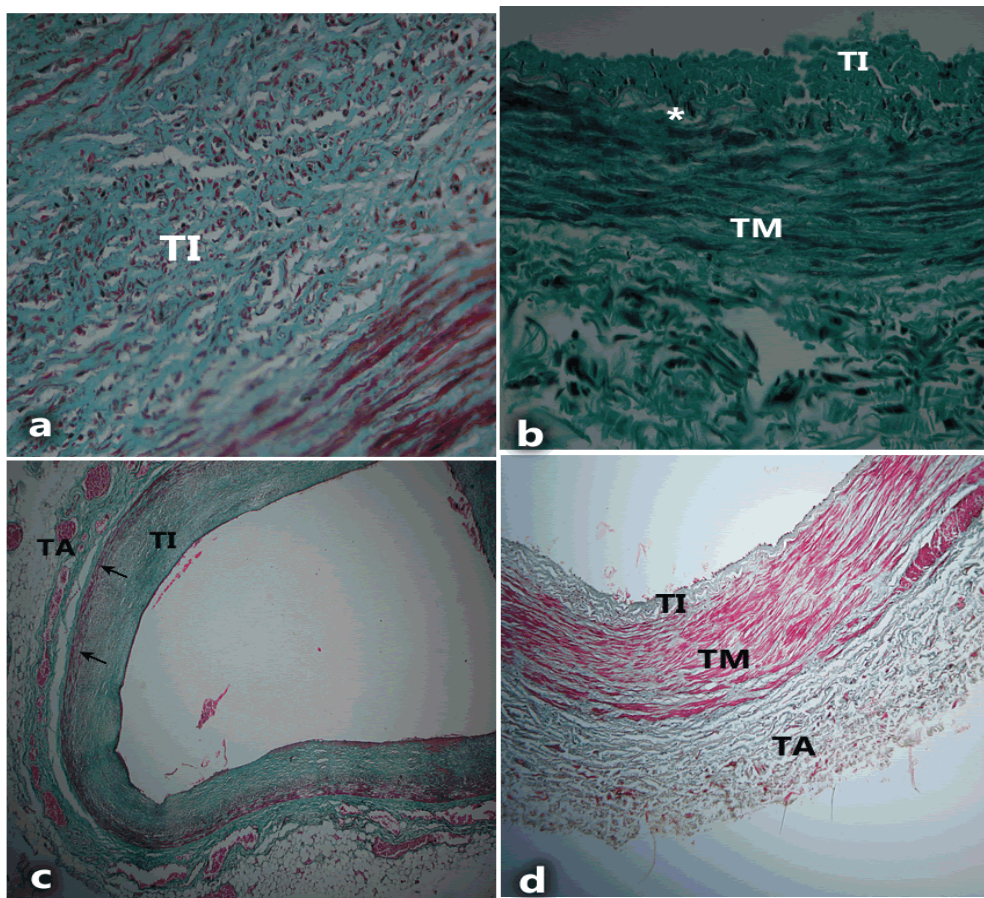

Figure 3 a - d: Histomorphological features of atherosclerosis in LAD of black Kenyans. TI = Tunica intima; TM = Tunica media; TA = Tunica adventitia. Mason's trichrome and Hematoxylin/Eosin. A - Hyper cellularity of intimal thickening in a 36 year old male. Note the abundance of cells of various sizes and shapes in TI x400. B - Disintegration of IEL, causing gaps (star) in a 62 year old female. *x 400. C -* Attenuation of tunica media in a 43 year old male. Note the very markedly thinned out smooth muscle layer (arrows) *x 40. D -* Thickening of the tunica adventitia (TA) making almost the same size as the tunica media (TM) in a 35 year old male. *x 100.*

Mural neovascularization

The other distinct feature was mural neovascularization. In 10 (18.5%) cases there was profuse proliferation of the vasa vasora in the tunica adventitia (Figure 4a). The vasa vasora invaded the tunica media, with the vessels running between the layers of smooth muscle in 3 cases (Figure 4b). In 2 cases of very severe intimal hyperplasia, vessels were noted in the tunica intima (Figure 4c).

Age and gender distribution

The mean age of those with features of atherosclerosis was 38.4 years (Range 6 – 62 years). Majority were aged below 50 years, with 25 (46.3%) being below 40 years. Two (3.7%) were aged below 10 years and 4 (7.4%) were teenagers. Only 15 (27.8%) were over 50 years old (Fig 6). There was a male predominance in all age groups with overall male: female ratio of 1.7:1. Male predominance was most pronounced between 31 – 60 years (Figure 5).
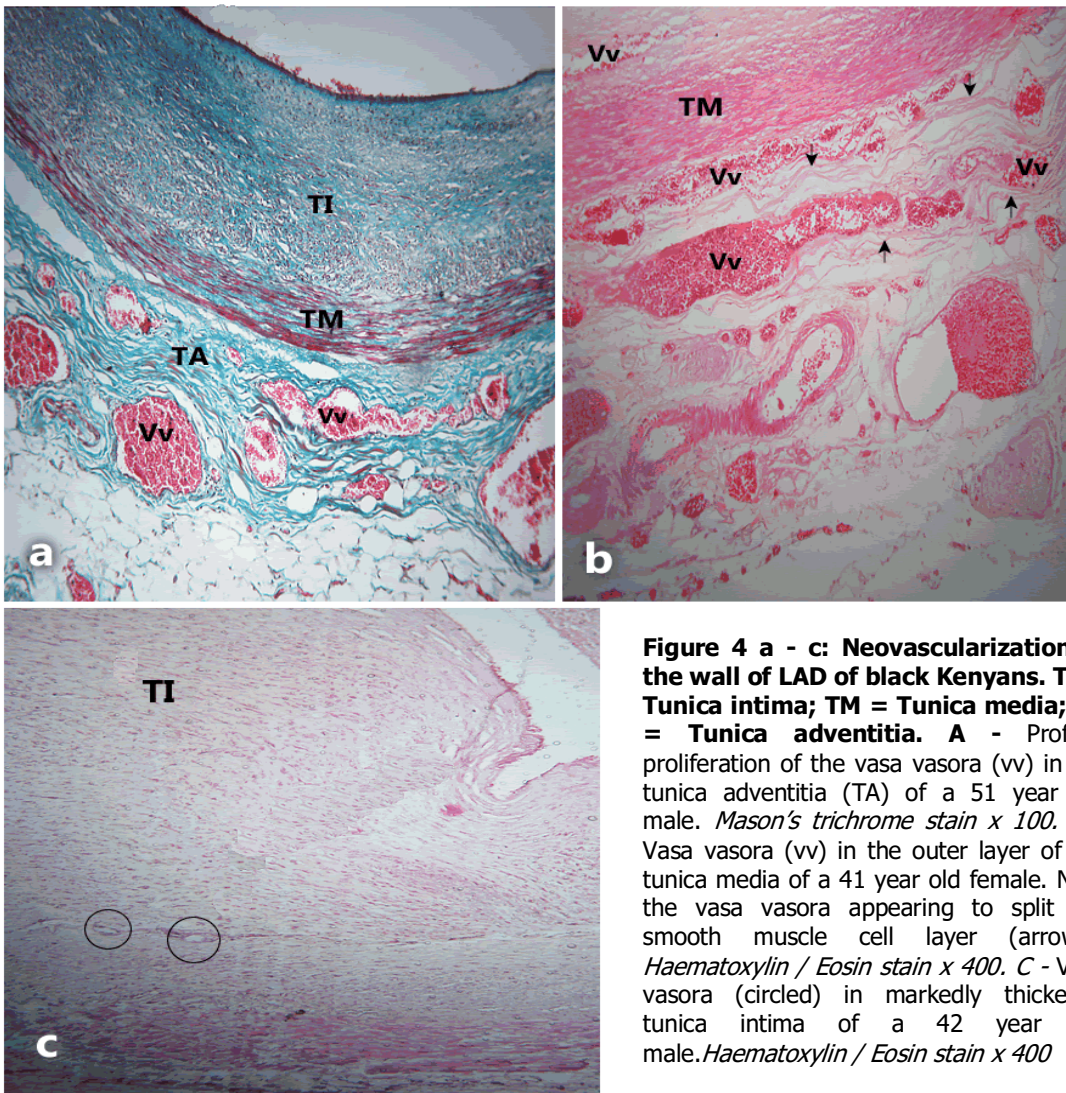

Figure 4 a - c: Neovascularization in the wall of LAD of black Kenyans. TI = Tunica intima; TM = Tunica media; TA = Tunica adventitia. A - Profuse proliferation of the vasa vasora (vv) in the tunica adventitia (TA) of a 51 year old male. *Mason's trichrome stain x 100. B -* Vasa vasora (vv) in the outer layer of the tunica media of a 41 year old female. Note the vasa vasora appearing to split the smooth muscle cell layer (arrows). *Haematoxylin / Eosin stain x 400. C -* Vasa vasora (circled) in markedly thickened tunica intima of a 42 year old male.*Haematoxylin / Eosin stain x 400*
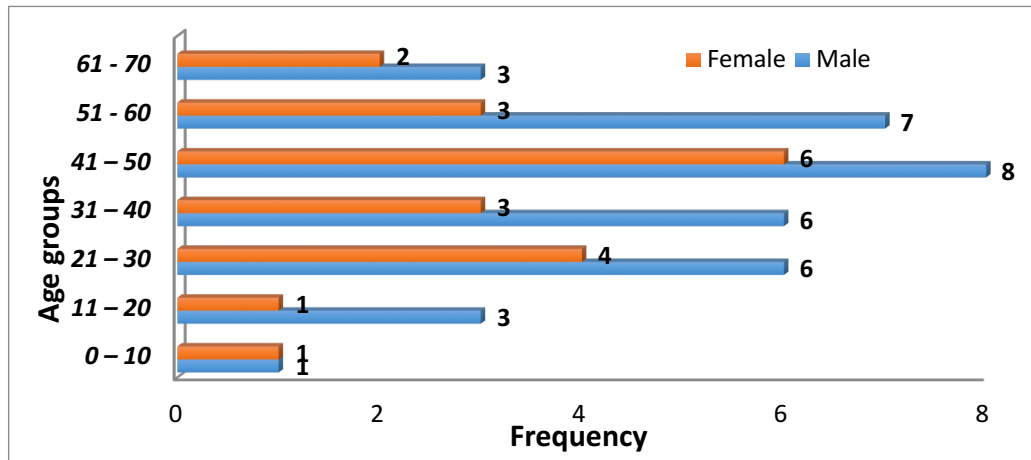
Figure 5: Age and gender distribution of cases with features of atherosclerosis

DISCUSSION

In the present study, features observed in the arterial wall which are consistent with various stages of atherosclerosis include intimal hyperplasia (Cizek et al., 2007; Subbotin, 2016); intimal hypercellularity (Libby et al., 2011; Bobryshev et al., 2011; Orekhov et al., 2014); disruption of elastic fibres in the internal elastic lamina (Koniari et al., 2011); degeneration of tunica media (Clarke et al., 2008; Bennett et al., 2016); adventitial thickening (Falk et al., 2009; Skilton et al., 2009; Stenmark et al., 2013; Wang et al., 2013) and mural neovascularization (Fleiner et al., 2004; Moreno et al., 2006; Hildebrandt et al., 2008). These observations revealed that all layers of the arterial wall are involved in atherosclerosis and confirmed our recent report that features of atherosclerosis do occur in the tunica adventitia (Ogeng'o et al., 2014b). This extensive involvement suggests that the disease is already established in the arteries and supports our earlier submission that atherosclerotic disease is a real problem which should no longer be considered uncommon among Kenyans (Ogeng'o et al., 2010a).

These features of atherosclerosis were found in 25.4% of the study population. This is much higher than 2% reported in the same population over 15 years ago (Saidi et al., 2002), comparable to 21% reported in Ethiopia (Maru, 1989) and 28.9% in Iran (Golshali et al., 2005) but lower than those reported from several Indo – Asian and Caucasian countries (Table 2).

This comparatively high prevalence is consistent with recent reports that myocardial infarction has become more common in the Kenyan population than it was 15 – 20 years ago (Ogeng'o et al., 2010a; 2014a; Bloomfield et al., 2016). In 20 (9.4%) of the total study population, there were atherosclerotic plaques. This is consistent with the prevalence of 2 – 11% reported for various populations (Al – Nozha et al., 2014). The variability revealed in Table 2 suggests geographical variations probably dependent on the profile of risk factors. The apparent increase in the Kenyan population may be attributed to the demographic and epidemiological transitions occurring in Kenya (Fengler, 2009; Gatongi et al., 2015). This implies that the disease may continue to rise unless deliberate control measures are instituted to contain it.

Table 2: Prevalence of coronary atherosclerosis in selected countries

Reference	Country	Percent Prevalence of coronary atherosclerosis
Al – Nozha et al., 2004	Saudi Arabia	5.5
Golshahi et al., 2005	Iran	28.9
Kim et al., 2013	Korea	48.5
Maru, 1989	Ethiopia	21
Saidi et al., 2002	Kenya	2
Tabib and Loire, 1993	France	34
Tuzcu et al., 2001	America	51.9
Vyas et al., 2015	India	73.5
Westerman et al., 2015	Germany	16.5
Current Study	Kenya	25.4

The most remarkable finding of the present study, however, was that in 2 (3.7%) cases (1 male; 1 female) the features were present the first decade and in 4 (7.4%) cases (3 males; 1 female) they were present in the second decade. This indicated that in 6 (11.1%) of the cases, the features were present in individuals aged 20 years and below. Features of atherosclerosis in the first decade have not been reported before. This precocious commencement is concordant with reports that exposure to risk factors may trigger atherosclerosis from an early age including in the prenatal stage (Matturi et al., 2003; Skilton, 2008; Frantz et al., 2012). Indeed, our earlier report indicated histomorphometric evidence of early onset of coronary heart disease (Ogeng'o et al., 2010b). The features observed usually correlate with risk factors (Cizek et al., 2007; Skilton et al., 2011, 2012). Their presence in the first decade is consistent with presence of risk factors in women of reproductive age in the Kenyan population (Mathenge et al., 2010; Joshi et al., 2014; Muchira et al., 2015) which would imply potential for prenatal exposure to these factors. Presence of features of atherosclerosis in the second decade, is on the other hand, consistent with many literature reports (Mc Gill et al., 2000; Cheng, 2001; Skilton et al., 2008; Modelli et al., 2011; Vyas et al., 2011). These findings imply that, like in other populations, control of atherosclerosis should commence early in life, including during the pre natal period.

In 46.3% of cases, the features were present in those aged 40 years and below, the so called young CAD. The high prevalence appears at variance with most studies which report 5 – 10% prevalence among South Asians and 1 – 2% in other ethnic groups (Aggarwal et al., 2016). Recent angiographic studies among Iraqis, however, revealed prevalence of 31% (Mohammad et al., 2015). The prevalence of 46.3%, however, appears concordant with intracoronary ultrasound studies which revealed over 50% prevalence of young CAD among asymptomatic young adults and children (Tuzcu et al., 2001). This suggests that the prevalence of young CAD shows geographical and perhaps ethnic differences, but fundamentally, it may be higher than currently perceived for most populations. These observations reveal wide diversity perhaps related to profile of risk factors and age distribution of the population. Pertinent to this suggestion are the findings that the implicated risk factors namely family history, male gender, hypertension, obesity, smoking, alcohol intake, diabetes mellitus (Yang et al., 2014; Albarqy et al., 2015; Aggarwal et al., 2016) are also rampant in the population (Mathenge et al., 2010; Ayah et al., 2013; Joshi et al., 2014; Muchira et al., 2015). Early onset coronary atherosclerosis is consistent with our recent report that over 30% victims of

myocardial infarction are aged 50 years and younger (Ogeng'o et al., 2010b). Control measures should therefore target these risk factors, commence in the prenatal period and be deployed continuously.

The male predominance in the current study is also consistent with previous reports that myocardial infarction is more common in males (Ogeng'o et al., 2010a; 2011), supporting the suggestion that male gender is a major non-modifiable risk factor (Albarqy et al., 2015). Among women, it is generally believed that endogeneous premenopausal estrogen protects them from coronary artery disease (Subbiah, 1993; Meyer et al., 2014; Romano et al., 2016). A remarkable finding of the current study, at variance with this assertion, is that there are substantial numbers of females affected in all age groups. This supports recent reports that CHD is a problem even in premenopausal women and should not be underestimated (Maas and Appelman, 2010). This unexpected presence in women may be related to the prevalence of risk factors for atherosclerosis among Kenyan women (Joshi et al., 2014; Muchira et al., 2015). These findings suggest that preventive measures should also target young women.

In conclusion, features of coronary artery atherosclerosis are present in over 25% of the population studied. They are present in all layers of the arterial wall and represent various stages of disease development. The disease starts in the first decade, affects young people, increases with age and though affecting more males, a significant proportion of young women are afflicted. This suggests that subclinical atherosclerosis is an established problem in the black Kenyan population. Proactive preventive measures including follow – up should commence early, and involve both men and women to mitigate the effects.

CONFLICT OF INTEREST: There is no conflict of interest in this study

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