REGIONAL HISTOMORPHOMETRY AND DISTRIBUTION OF ANDROGEN RECEPTOR IN THE COMMON CAROTID ARTERIES AMONG KENYANS

Thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Human Anatomy of the University of Nairobi

By

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DECLARATIONS

I hereby declare that this thesis is my original work and has not been presented elsewhere for approval and examination.

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DEDICATION

To my wife Catherine Nyokabi

Daughter Casey Namukonga

To my mum Catherine Khasiala

To my friends and colleagues

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ABBREVIATIONS

AR..... Androgen receptor

AR+..... Androgen receptor positive cells

CA..... Carotid Artery

CIMT..... Carotid Intimomedial thickness

CVD..... Cardiovascular Disease

EC..... Endothelial cells

EEL..... External elastic lamella

IEL..... Internal elastic lamella

IgG..... Immunoglobulin Gamma

IMT..... Intimomedial Thickness.

Is..... Intimal segment

KNH..... Kenyatta National Hospital

Ms.....Medial segment

MT.....Masons Trichrome

SPSS..... Statistical Program for social sciences

TCC..... Total cell count

VSMC..... Vascular Smooth Muscle cells

WE..... Weigert Elastin

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SUMMARY

Background: The carotid intimomedial thickness is a predictor of atherosclerosis in other vascular beds and is useful in cardiovascular risk stratification. Atherosclerosis is reported to be worse in males and seems to increase with age. There have been reports that androgens are protective while other reports suggest they are bane. Expression distribution of androgen receptors in the carotid intimomedial thickness may help explain the role of androgens in development and progression of atherosclerosis. The relationship between the androgen receptors and the carotid intimomedial thickness is unknown.

Hypothesis: There are segmental, age and gender differences in the carotid intimomedial thickness which are influenced by presence of androgen receptors.

Objectives: To describe the distribution of androgen receptors in the common carotid artery intima and media and relate them with the carotid intimomedial thickness among Kenyans.

Study design: Descriptive crossectional study

Setting: Department of Human Anatomy, University of Nairobi.

Materials and Methods: Materials for this study were obtained from twelve (6 males and 6 females) common carotid arteries during autopsy within 48 hours of demise at the Chiromo funeral parlour. Three millimetre samples from the proximal, middle and distal thirds of artery segments were collected. They were fixed in 10% formaldehyde solution immediately and routinely processed for light microscopy. Another set of slides were stained using anti-androgen receptor monoclonal antibody (AR 318). All prostate samples used as positive controls were immunopositive for androgen receptors. The total cell count and the number of stained cells for each designated vessel zone were assessed in the three sections in four visual fields per section at 400 x magnification using a Zeiss® photomicroscope. The carotid intimomedial thickness was measured. The presence and distribution of androgen receptors

were observed and described. The intimomedial thickness was measured using the Scion Image Multiscan® software. The data collected was analysed using SPSS® version 18 for Windows® for means and variances.

Results: The mean age of the cases was 28±19yrs. Mean carotid intimomedial thickness was 0.86±0.22 mm (Male: 0.97±0.22; Females 0.77±0.06), p=0.05. Mean proximal, middle and distal intimomedial thickness were 0.86+0.26 mm, 0.84+0.28mm and 0.90+0.35mm respectively. Carotid intimomedial thickness increased with age; 0.5±0.16mm, 0.87±0.24mm and 1.21±0.36 mm for the age groups 0-20yrs, 21-40yrs and 41-60yrs respectively (p=0.035). Androgen receptors were not detected in the 12 samples tested.

Conclusions:

The common carotid artery is an elastic artery with a well-developed tunica intima and does not display androgen receptors. Carotid intimomedial thickness increases distally, with age and is higher in males. The carotid intimomedial thickness is not influenced by the presence of androgen receptors.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The common carotid artery (CCA) originates from the brachiocephalic trunk and aortic arch on the right and left respectively (Sinnathamby, 1999; Standring et al., 2004). This artery supplies the brain, extracranial structures in the head, and neck region and it can be divided arbitrarily into three parts: proximal, middle and distal (Watson and Silverstone, 1939; Skandalakis et al., 2004). The proximal region extends from the origin to the sternoclavicular joint, while the middle section is located in the neck between the sternoclavicular joint and the 2nd cervical vertebra. The distal part extends from the 2nd cervical vertebra to the carotid bifurcation at C3/4. It is a common site of atherosclerosis (Bo et al., 1989). Further, it increases with age and it is more common in males than premenopausal females (Hansen et al., 1995; Samijob et al., 1998). The reason for this regional gender and age preference are largely unclear. Intrinsic vascular factors that may explain these differences remain largely underreported. These include carotid intimomedial thickness and expression of androgen receptors (Tell et al., 1989).

The structure of the mammalian CCA varies according to species some revealing a muscular media (Kimani, 1983; Gabella et al., 2005), while others reveal an elastic structure (Pinto et al., 1998; Carallo et al., 1999; Orsi et al., 2006; Parchami et al., 2009; Parchami and Dehkordi, 2011). There are segmental differences in structure of the sheep, goat and giraffe carotid artery (Kimani, 1983; Parchami et al., 2009). These regional changes are influenced by the hemodynamic differences due to the length of the neck and body posture (Kimani, 1983). All artery tunics show age related changes in structure (Toda et al., 1980). These include intimal thickening, fragmentation of the internal elastic lamina, elastic tissue

fragmentation, collagen deposition in the tunica media (Futterman and Lemberg, 2003; Lee and Oh, 2010; Kotsis et al., 2011). Although these changes occur in virtually every artery, there are differences in the age of onset and degree of change experienced by different arteries (Toda et al., 1980). Generally, males have a thick intima and media and usually age faster than females (Lakatta and Levy, 2003). These gender differences in the vessel structure have been related to the effects of androgens (Sader and Celermajer, 2002).

The exact role of androgens in development and progression of atherosclerosis is controversial; with some studies showing protection (Bonnel et al., 1941; Alexandersen et al., 1999) while others demonstrate detriment (Liu et al., 2001; Lawlor et al., 2004; Ramirez et al., 2007). Effects of androgens are mediated through androgen receptors (AR) (Liu et al., 2003). These receptors have been demonstrated in cultured vascular macrophages, endothelial cells (EC), fibroblasts and smooth muscle cells (Kimura et al., 1993; Liu et al., 2005; Rexrode et al., 2008; Sieveking et al., 2010), and show gender dichotomy in expression. Males exhibit a four-fold higher expression AR than females (McCrohon et al., 2000; Ng et al., 2003).

Post-mortem studies have elucidated different numbers of AR in the coronary arterial wall, inversely relating them to early atherosclerosis (Liu et al., 2005). Relating the distribution and number of androgen receptors to the carotid structure and CIMT would give some insight into the role of androgens in the gender differences in development and progression of atherosclerosis. The pattern of expression of androgen receptors in the carotid arterial wall is unknown. Expression of androgen receptors if related to the vessel segment, age and gender may explain the gender and regional differences in atherogenesis (McGill and Sheridan, 1981; McGrath et al., 2008). This study therefore describes the segmental structure,

histomorphometry and androgen receptor expression in the carotid artery in males and females of different age groups.

1.2 Literature Review

The common carotid artery is a common site of atherosclerosis. This preponderance is related to the changes in its intimomedial thickness (Sader et al., 2001; Liu et al., 2003). Furthermore, the intimomedial thickness may show age, gender, segmental differences related to the distribution of androgen receptors in the different mural layers. Mammalian CCA wall is composed of three histological layers namely the tunica intima, tunica media and tunica adventitia (Kimani, 1983; Parchami et al., 2009). The tunica intima is composed of a single layer of endothelial cells and the subendothelial zone (SEZ), a region just below the endothelial surface but on the adluminal side of the IEL (Timmins et al., 2010). The tunica media in some mammals is preponderantly elastic, with concentric elastic lamellae and interposed smooth muscle cells and collagen (Orsi et al., 2006; Parchami et al., 2009). Other mammals have muscular CCA tunica media (Kimani, 1983). Arterial adventitia on the other hand is fibroelastic (Wang et al., 2010).

1.2.1 Carotid Intimomedial thickness

Although CIMT is not yet routinely measured in clinical practice, its predictive value regarding cardiovascular complications has been established, giving it a potential role in future for cardiovascular disease risk stratification and primary prevention (Hodis et al., 1998; Greenland et al., 2000; Jarauta et al., 2010). The human carotid intimomedial thickness is about 0.8 to 0.91mm, measured using ultrasound (Jadhav and Kadam, 2001; Okeahialam et al., 2011). It is 1.049mm and 0.55mm in Americans and Nepalese respectively (Crouse et al., 1995; Sharma et al., 2009).

1.2.2 Age and gender related changes in carotid histomorphometry.

Aging changes in muscular arteries include; intimal thickening, elastic tissue fragmentation, and smooth muscle hyperplasia (Roach, 1970; Nakagami and Morishita, 2008). The internal

elastic lamina (IEL) becomes less organized with aging (Gross et al., 1934). Notable changes in the tunica media include increased collagen and smooth muscle deposition, thickening and subsequent stiffness (Cameron et al., 2003). The external elastic lamina (EEL) thickens with age (Kawasaki et al., 1987). The tunica adventitia ages by collagen deposition and elastic tissue loss (Gutterman, 1999; Wang et al., 2010). These changes occur earlier in males than in females before menopause, levelling afterwards (Kannel et al., 1976; Colditz et al., 1987; English et al., 1997; Futterman and Lemberg, 2003). This gender specific age changes have therefore been related to the effects of sex hormones (Juul and Skakkebaek, 2002; Anderson and Pepine, 2007). The age of onset of these changes in the common carotid artery is not described. Segmental intimomedial thickness in the carotid artery, aging changes and the relationship with the number and distribution of vascular AR, in the common carotid artery in both genders is unknown.

1.2.3 Androgens and androgen receptors in the carotid artery

The marked sexual dimorphism that exists in IMT and human cardiovascular diseases has led to the concept that androgens have deleterious effects and exacerbates the development of cardiovascular disease in males (Perusquia and Stallone, 2010; Parchami and Dehkordi, 2011). The carotid luminal diameter is wider in females than males; the converse is true for wall thickness (Parchami and Dehkordi, 2011). Androgens have traditionally been regarded as the proximate cause underlying this male disadvantage (Wu and Eckardstein, 2003). Although the CIMT and atherosclerosis increase with age, serum levels of endogenous androgens decrease with age (Bernini et al., 1999; Jarauta et al., 2010). If the effects of androgens persist despite their lower levels in aging, it is probable that there is up regulation of androgen receptors in the elderly. The older individuals with wider CIMT must have more androgen receptors in the carotid mural layers. The role of androgens in the age related

worsening of intimomedial thickness and subsequent development of atherosclerosis is however unknown. Androgen receptors are expressed in the arterial wall of mammals including man (Fujimoto et al., 1994; Hanke et al., 2001; Rexrode et al., 2008; Sieveking et al., 2010). Expression and numbers of these receptors in the coronary artery have a linear correlation with protection from atherosclerosis (Liu et al., 2005). Furthermore, treatment of rats with testosterone is associated with inhibition of neointimal plaque formation and increased expression of the AR mRNA after endothelial injury (Hanke et al., 2001; Li et al., 2008). Androgens have also been shown to stimulate proteoglycan proliferation and elongation of vascular smooth muscles, promoting atherosclerosis (Hashimura et al., 2005). However, other studies (Somjen et al., 1998) have shown that androgens inhibit vascular smooth muscle cell (VSMC) proliferation, thereby protecting against atherosclerosis. Independently, androgens have also been found to up regulate their receptors (Ma et al., 2005). Over expression of AR in endothelial cells confers them with increased androgen sensitivity (Sieveking et al., 2010). Vascular AR may have a cellular or zonal preference and their expression may be related to the histomorphometry of the artery, especially the intimomedial thickness. This information could predict the role these receptors in increased intimomedial thickness and subsequent development of atherosclerosis.

1.3 Justification

The common carotid artery is prone to atherosclerosis and thromboembolic phenomena which are a common cause of stroke and mortality especially in males (Bösel et al., 2010; Hart and Benavente, 1999). When exposed to the same lifestyle, the male predominant atherosclerosis, thromboembolic phenomena, arterial wall stiffness, dissection and aneurysm in the CCA may be related to the carotid intimomedial thickness and effects of sexual hormones (Lerner and Kannel, 1986; Ahimastos et al., 2003). While the benefits of

oestrogens in sparing arteries from atherosclerosis are largely known (Tracy et al., 1966; Sack et al., 1994), controversy still exists as to the role of androgens in increasing CIMT and subsequent development of atherosclerosis (Bernini et al., 1999; McGrath et al., 2008). This knowledge is relevant now in an attempt to resolve the role of androgens in atherogenesis in both genders, as current research is reviewing complementary theories of atherogenesis and management of atherosclerosis.

1.4 Significance of the study

The common carotid artery is the major source of blood to the brain, head and neck region (Sinnathamby, 1999). It is frequently plagued by atherosclerosis (Duvall and Vorchheimer, 2004). Mural factors predisposing the common carotid artery to atherosclerosis include its carotid intimomedial thickness and androgen receptor distribution (Ramirez et al., 2007; Jarauta et al., 2010). Carotid intimomedial thickness in sheep varies with age and shows segmental and age differences (Parchami and Dehkordi, 2011). The relationship between CIMT and androgen receptor distribution in the human carotid artery is unknown. This study therefore describes the segmental histomorphology and dimensions of the carotid artery in humans. It also relates the expression of androgen receptors to the carotid intimomedial thickness. This information is useful in understanding aging of the carotid arteries, patterns of atherogenesis in both gender and the role of androgens in atherogenesis.

1.5 Study Question

Epidemiological studies have shown there is a striking gender difference in cardiovascular disease with men having higher rates of clinical events than women (Kalin and Zumoff, 1990). Gender patterns in the prevalence and onset of carotid atherosclerosis may be related to its histological structure and dimensions (Rosfors et al., 1998). Less is known regarding the relationship between CIMT, an established cardiovascular risk factor and the presence of AR in the carotid artery. Prior to this, no study has been designed to evaluate the morphologic appearance of the non-pathologic human carotid and its relationship to the presence and distribution of androgen receptors.

1.6 Hypothesis

Alternate hypothesis

There are segmental, age and gender differences in the carotid intimomedial thickness which are influenced by presence of androgen receptors.

1.7 Objectives

1.7.1 Broad objective

To describe distribution of androgen receptors in the common carotid artery intima and media and relate them with the carotid intimomedial thickness among Kenyans.

1.7.2 Specific Objectives

With respect to the common carotid artery among Kenyans;

1. To describe the histomorphometry
2. To describe the age and gender differences in histomorphometry.
3. To describe the segmental differences in carotid intimal medial thickness.
4. To demonstrate androgen receptors in different mural layers.

CHAPTER TWO: MATERIALS STUDY METHODS

2.1 Study design and setting

A descriptive cross-sectional study carried out at the Department of Human Anatomy University of Nairobi, between March and June 2011.

2.2 Ethical considerations in the study

All protocols were carried out according to a study proposal approved by the UON/KNH-Ethical and Research committee. Relatives of the deceased gave consent for use of the carotid artery samples for this particular study (Appendix 1). The names and identities of the deceased were not used during sample collection. Three millimetre sections from the proximal, middle and distal segments of the common carotid artery were harvested for histology during autopsy. Remnants of the histologic material that were not used in the study were decently buried at Langata cemetery, Nairobi.

2.3 Sample size and distribution

The number of samples was calculated according to formula provided by Eng (2003).

$$N = \frac{4\acute{o}^2 (Zcrit)^2}{D^2}$$

In the above formula, N was the sample size required. It was assumed that there was a standard deviation (ó) of 2.5 for each variable in each group, and a minimum expected difference (D) between the means was 2.5 (McRobb et al., 2009). Zcrit of 1.960 based on significance criteria (P-value) of 0.05 was used.

Accordingly;

$$N = \frac{4 \times 2.5 \times 2.5 (1.96)^2}{2.5^2}$$

N = 12.36 Approx. 12

Twelve common carotid arteries from 6 males and 6 females were used in this study. The samples were divided into two groups; male and female; in three age groups (0-20, 21- 40 and 41-60 years). Among females, the first two age groups correspond to the reproductive age groups while the last were postmenopausal.

Selection criteria

2.3.1 Inclusion Criteria

Common carotid artery samples that were used met all listed conditions;

1. Cases whose relatives gave consent to participate in the study.
2. Individuals without gross defects and atherosclerosis.

2.3.2 Exclusion Criteria

The following individuals were excluded from the current study

1. Individuals with major neck trauma damaging the carotid arteries.
2. Patients who died from hypertension or diabetes.

2.4 Controls

Control samples were used during the immunohistochemical staining of androgen receptors for quality control. Both positive and negative controls were used.

a. Positive control

Three post-mortem prostates samples from 3 different males were harvested within 48hrs of demise were used as positive controls for androgen receptor immunohistochemical staining (Bayer-Garner et al., 1999; Rocha et al., 2000).

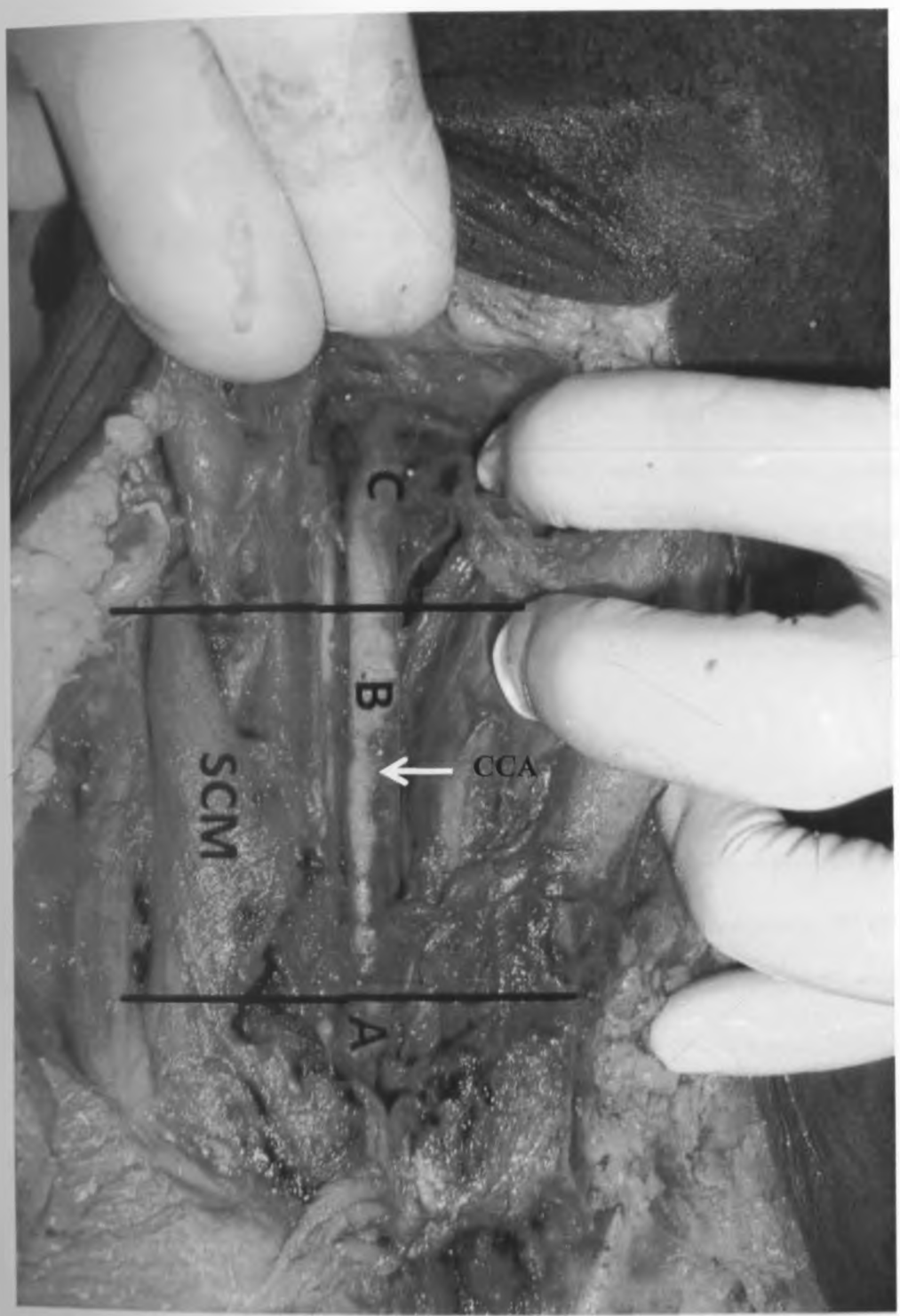
b. Negative control

The omission of the primary antibody and substitution with dilution solution alone served as a negative control (Gallardo et al., 2009).

2.5 Accessing the common carotid artery

A skin incision was made in the neck along the impression of the anterior border of sternocleidomastoid (SCM) from the angle of the mandible to the sternoclavicular joint (Figure 1). The skin, investing cervical fascia were separated and retracted exposing the contents of the carotid sheath. The common carotid artery was dissected from the root of the neck to the bifurcation point and divided into three proportional segments; A (Proximal), B (Middle) and C (Distal) (Figure 1). Three millimetre tissue samples of the whole vessel wall were measured and harvested from the proximal, middle and distal segment including the bifurcation in cases less than 72 hours from the time of demise.

Figure 1: Reflected skin to expose the CCA



A macrograph showing the reflected skin, a separated sternocleidomastoid (SCM) muscle exposing the common carotid artery. Arterial segments A, B and C were taken, A (Proximal); B (Middle); C (Terminal).

2.6 Histological methods used

2.6.1 Tissue processing for light microscopy

The 3mm sections harvested from the three regions described above and 3mm prostatic tissue were used. They were fixed in 10% formal saline for 24 hours, immediately after harvesting and dehydrated in increasing concentrations of ethyl alcohol starting with 70% ethanol up to absolute alcohol for one hour each. Toluene was used as a clearing agent for 1 hour before infiltrating them with paraffin wax for 12 hours at 56^0c. The tissues were blocked into paraffin waxed blocks. Seven micrometer thick sections were then cut using a Leitz Wetzlar® sledge microtome. They were floated in warm water and thereafter mounted and dried in hot air oven at 40^0C for 12 hours. Dewaxing was then done using xylene for 5 minutes after which the tissues were rehydrated using ethyl alcohol for 5 minutes and then with decreasing concentrations of alcohol from absolute alcohol to 70% alcohol for 5 minutes each.

2.6.2 Staining Techniques

Haematoxylin and Eosin was used to study the general tissue characteristics. The Haematoxylin stains the nucleus while the Eosin counter stains it by staining the cytoplasm and connective tissue with varying intensities (Drury et al., 1967). Masson's Trichrome was used to study the cytoarchitecture and connective tissue of the carotid tunics. Some sections were stained with Weigert's Resorcin Fuschin then counterstained with van Gieson stain to demonstrate the elastic component of the vessels. The slides were examined under Leica® light microscope at magnification x40, x100 and x400. The structure of the tunica intima (TI), media (TM) and adventitia (TA) were noted. The information made was recorded on data sheets.

2.7 Immunohistochemistry technique

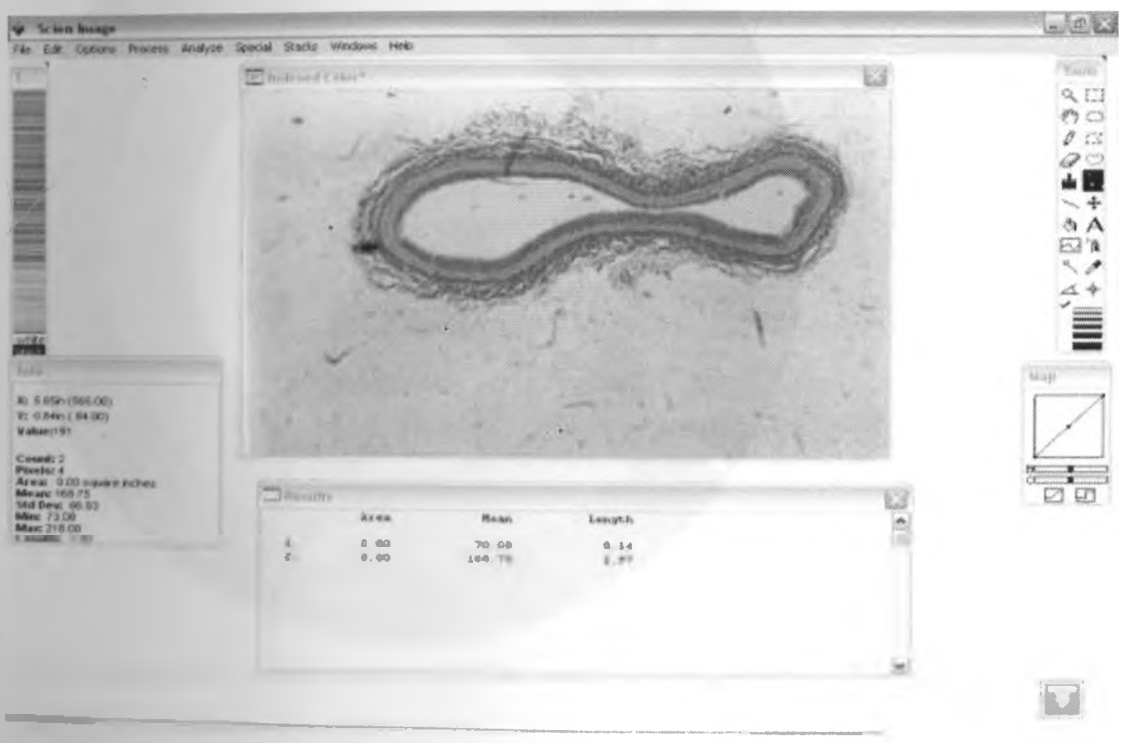
Paraffin sections 3μm were cut and mounted on previously charged and coded microscope slides. These included a positive control (prostate sample) and the test section (carotid sample). All prostate samples were immunopositive for androgen receptors. The sections were fixed in the bond-max covertiles. Respective labels were then affixed to the slides and the racks slotted into the machine for autostaining. A high amplification, biotin free detection system optimized for use on the bond system was used for staining. Autoimmunostaining occurred as follows; dewaxing was done by a commercially available dewax solution® for 15 minutes. Specimens were incubated with hydrogen peroxide for 5 minutes to quench endogenous peroxidase activity. Antigen retrieval was done by microwaves at 98^0c for 20 minutes at a Ph of 9.0 (Janssen et al., 1994). They were then incubated with the primary antibody (AR – 318) for 15 minutes then rinsed using bond wash buffer (Sajjad et al., 2004). Post primary IgG linker reagent was applied to the sections for 8 minutes to localize the antibody, followed by a buffer wash. Sections were then incubated with bond polymer for 8 minutes then rinsed using bond wash buffer. Sections were incubated for 10 minutes in 3, 3'-diaminobenzidine tetra hydrochloride (DAB) (Sigma) which forms a brown precipitate with the complex so as to aid visualization. Harris haematoxylin counterstaining was done for 5 minutes to allow visualization of the nuclei (Merck, Poole, Dorset, UK). At the end of the staining the racks were dislodged from the staining chamber and the stained slides fitted into a staining rack. The sections were dehydrated into two changes of alcohol, and cleared in three changes of xylene. The slides were then mounted in DPX. Slides were examined by the principal investigator and a pathologist who were unaware of the gender and ages of the individuals. The total cell count (TCC) and the number of AR stained (AR+) cells for each

designated vessel zone were assessed in the three sections in three visual fields per section at 400 x magnification using a Zeiss® photomicroscope.

2.8 Morphometry

Slides were photographed using a Zeiss® digital photomicroscope at magnifications of x40 and x100 for analysis. Every fifth slide from each gender and age group were picked and measurements taken as described below. Analysis of the photographs was done using Scion Image™ Multiscan software (Scion Corporation, Frederick, Maryland) after calibration accurate to 0.01 mm, using ruler measurements of the histological slides [Figure 2] (Nakashima et al., 2002).

Figure 2: Scion Image analyser



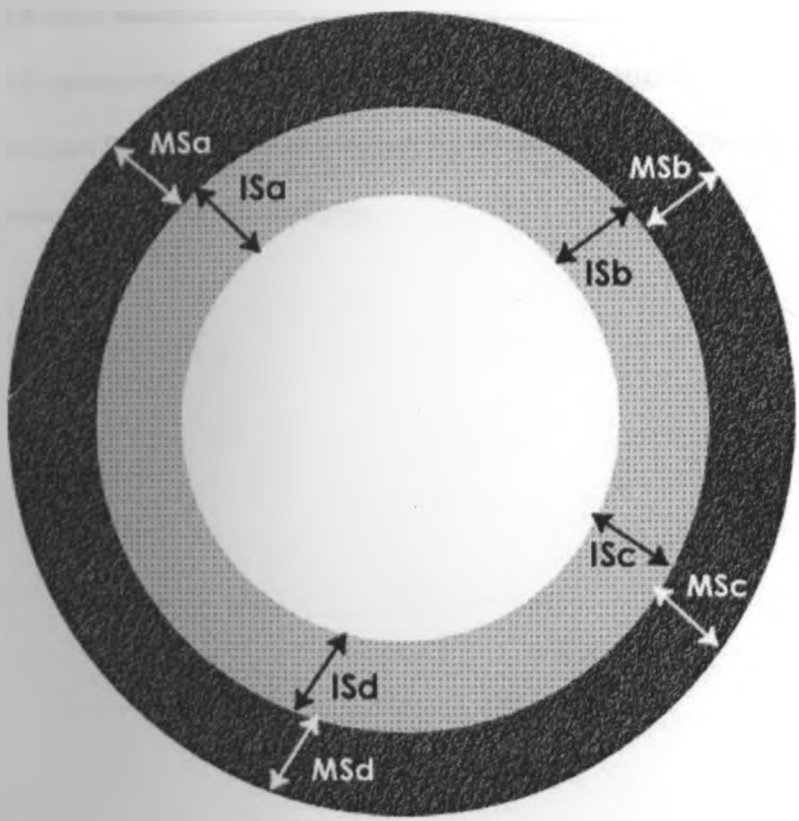
A digital ruler used to measure the various parameters of the CCA. Adopted from Nakashima et al., 2002.

The following parameters were determined;

2.8.1 Intimal thickness

Intimal extent was taken as the region extending from the lumen to the internal elastic lamina separating the tunica intima and the tunica media (Fernie and Lamb, 1985). Four random points were selected and the average length was calculated (Figure 3).

Figure 3: Segments of the vessel wall that were measured.



Intimal sections (IS), medial section (MS) of the vessel wall were measured at four random points (a-d). Adopted from Nakashima et al., 2002.

2.8.2 Medial thickness

Medial extent was taken as the region extending from beneath the thin tunica intima to the elastic fibres separating the medial and adventitial tunics. Four random points were selected and the average length was calculated.

2.8.3 Intimal-medial thickness

Carotid intimomedial thickness was taken as the region covered by both the tunica intima and the tunica media.

2.8 Inter observer Error

All measurements were done by the principal investigator and an assistant. The difference between the two measures were not significant (p=0.656). The true measure was taken as the mean between the two readings.

2.9 Data handling and management.

The data collected was tabulated, coded and analysed using a statistical program SPSS® version 18 for Windows® 7 (SPSS Inc. Chicago Illinois, 2010). The intimomedial thickness was determined for the different age and gender groups. Tables and charts were used to illustrate the findings. The F test was used to determine significance. A p value of $P<0.05$ was considered significant (Eng, 2003).

CHAPTER THREE: RESULTS

There were 6 males and 6 females with a mean age of 28±19yrs (28±20yrs for females; 29±20males). The youngest sample was from a 1yr old while the oldest was 60 yrs old.

The mean sample collection time after demise was 18hrs. The common carotid arterial wall comprised of three histological layers namely; tunica intima, tunica media and tunica adventitia (Fig. 4A).

The tunica intima comprises of an endothelium of elongated flattened cells with prominent, darkly stained nuclei, pale cytoplasm and the SEZ (Fig. 4B). The SEZ comprises of loosely arranged collagen interspersed with elastic and spindle shaped oval nucleated cells akin to smooth muscle cells (Fig. 4A-D). Smooth muscle cells were oriented axially (Fig. 5 D). In some places it thickened to form focal intimal thickening (Fig. 4C). A wavy internal elastic lamella (IEL) separated the intima from media (Fig. 4E).

The tunica media was the thickest layer constituting about 80% of the carotid wall (5D). It was predominantly elastic, with 20 to 35 concentric wavy elastic lamella interspersed with collagen and smooth muscle cells (Fig. 5A, Fig 5B). The elastic lamellae decreased to 25-30 in the middle and 20-22 in the distal sections. In some sections the media displayed a sub-intimal zone of smooth muscle cells densely packed in the luminal part of the tunica media (Fig 5C). Smooth muscle cells were arranged in layers; circumferential and longitudinal patterns between the elastic lamellae (5A). Smooth muscle cells increased in the tunica media distally. Collagen fibres were loosely arranged in circular, oblique and transverse directions around the vessel wall (Fig 5C). The amount of collagen fibers in the tunica media increased with age. About eighty percent of the vessel wall volume was occupied by the tunica media. The tunica adventitia is predominantly fibroelastic, with interspersed smooth muscle cells

(Fig. 6A), in two layers, the inner compact and the outer loose zone (Fig 6B). Zonation of the tunica adventitia was observed in the middle and distal sections of the artery. The elastic fibers in the adventitia were arranged in a circumferential pattern. It also had prominent vasa vasora (vv) and nervi vasora (nv) [Fig. 6C]. The vasa vasora extended into the media and intima in some sections (Fig 10C).

Figure 4: General structure and tunica intima of the CCA

A: A photomicrograph of a section of the CCA showing three layers namely; tunica Intima (TI), Tunica Media (TM) and Tunica Adventitia (TA) (Magnification x40, Masons trichrome stain).

B: A photomicrograph of a section of the CCA showing the tunica intima comprised of squamous endothelial cells (EC) and the SEZ. The SEZ had collagen fibers (starred) and spindle shaped oval nucleated cells akin to SMC (Magnification X400, Masons trichrome).

C: A photomicrograph of a section of the CCA showing Focal intimal thickening (DIT) (Arrowed) in the SEZ. This zone was composed of loosely arranged collagen interspersed with smooth muscle cells (Magnification X40, Masons trichrome).

D: A photomicrograph of a section of the tunica intima of the CCA with intimal hyperplasia (IH), showing a hyperplastic SEZ comprised of loosely arranged elastic fibers and smooth muscles (Arrowed). The smooth muscle cells are oriented longitudinally away from the endothelium.

E: A photomicrograph of a section of the CCA showing a wavy IEL (Black arrow), separating the intima from media. Concentric circumferential elastic lamellae in the tunica intima are also shown by the green arrows (Magnification X400, Weigert Elastin).

Figure 4: Photomicrograph of the general structure and tunica intima of the CCA

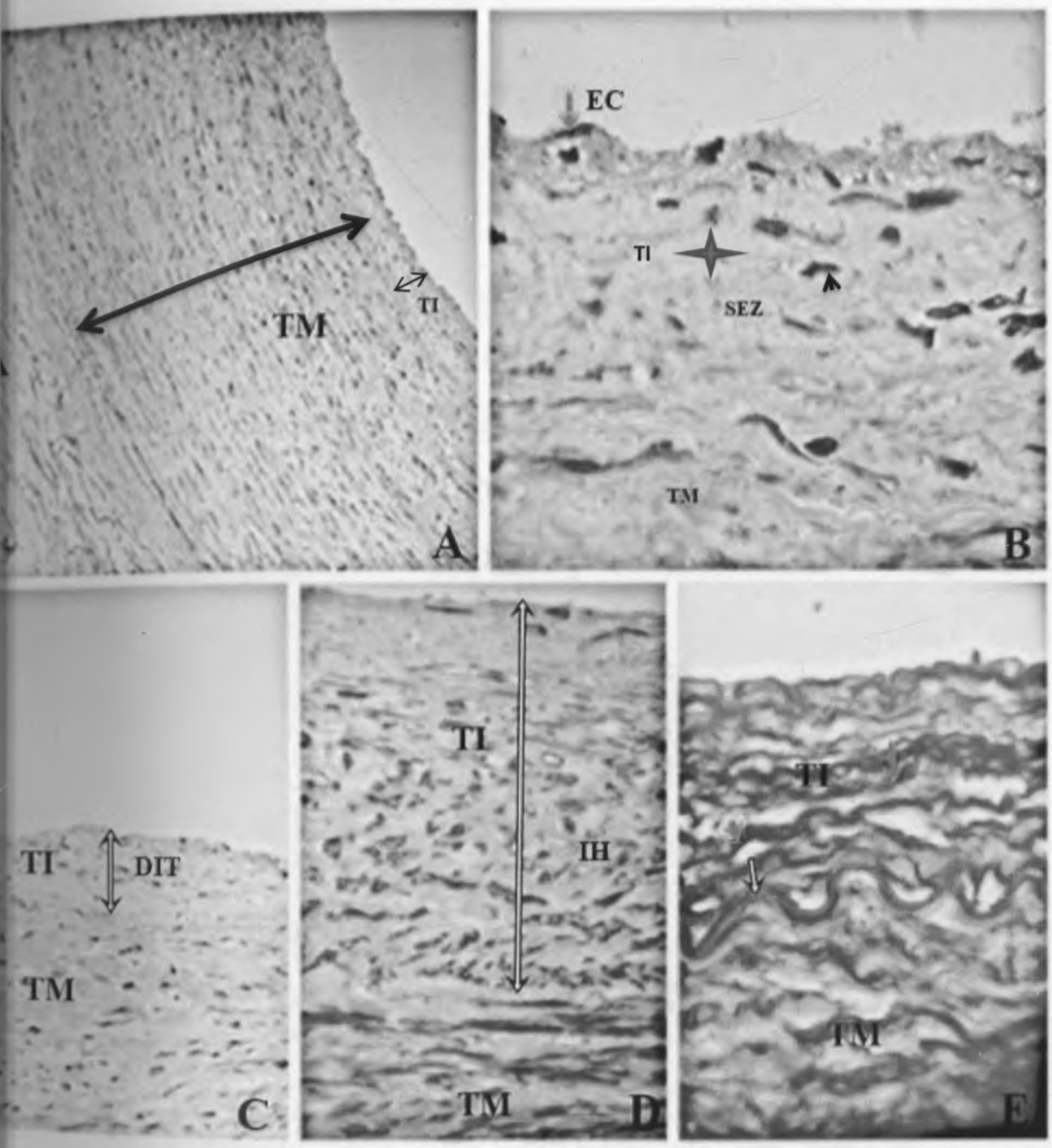


Figure 5: The tunica media of the common carotid artery

A: Photomicrograph of the common carotid artery tunica media. Concentric elastic lamellae are shown (white arrow), they are interspersed with collagen (starred) and circumferential smooth muscle cells [Red arrows]. Some smooth muscle cells are arranged longitudinally [blue arrow] (Magnification X100, Mason Trichrome).

B: Photomicrograph of the CCA media showing the concentric elastic lamellae (red arrow) in the tunica media (Magnification X100, Weigert Elastin).

C: Photomicrograph of the CCA showing circumferential smooth muscle cells (Starred) densely packed in the inner part of the tunica media to form the sub intimal zone (Magnification X400, Masson Trichrome).

D: The tunica media comprised of about 80% of the vessel wall (Magnification X40, Haematoxylin Eosin).

Figure 5: Photomicrograph of the tunica media of the common carotid artery

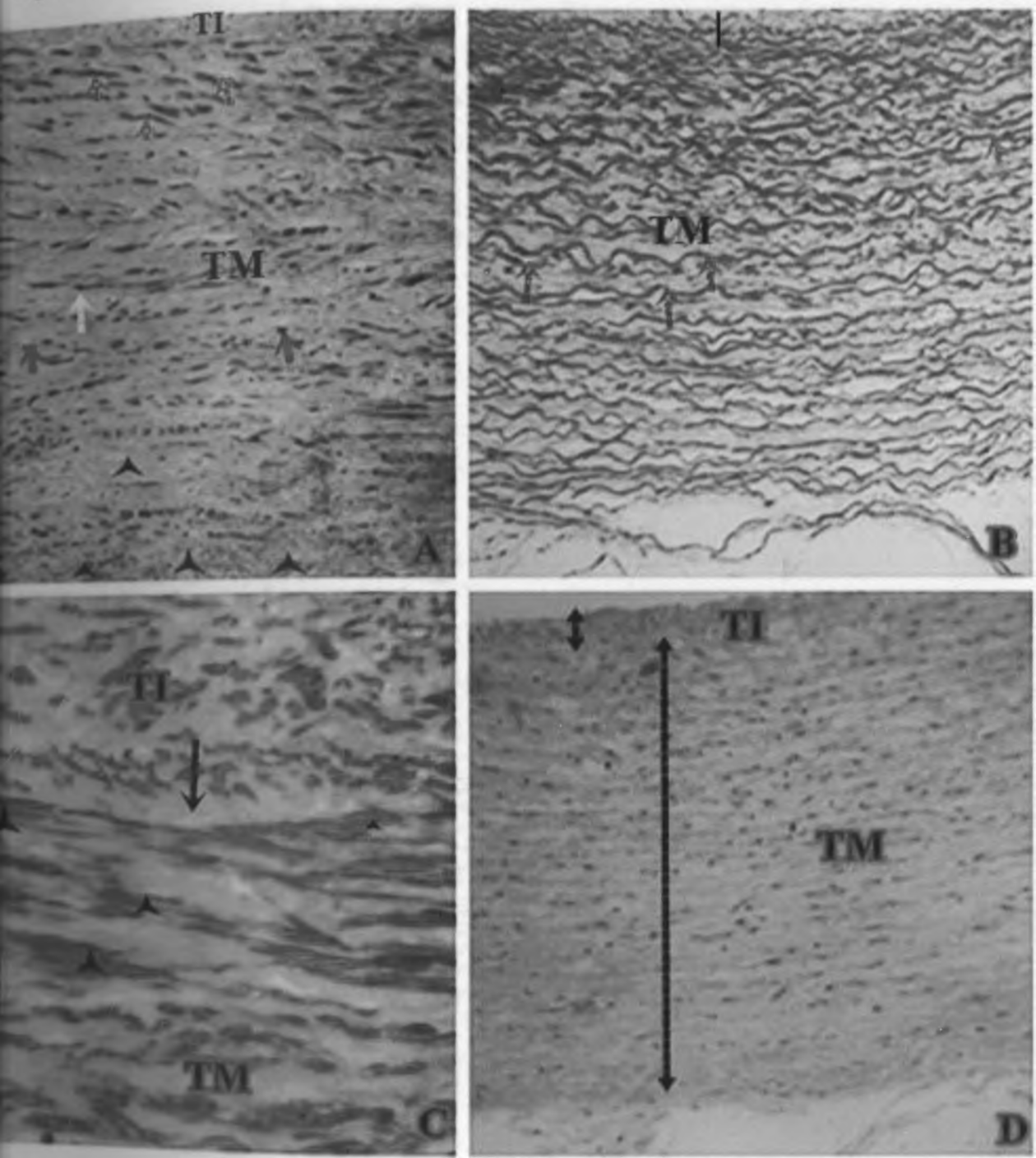


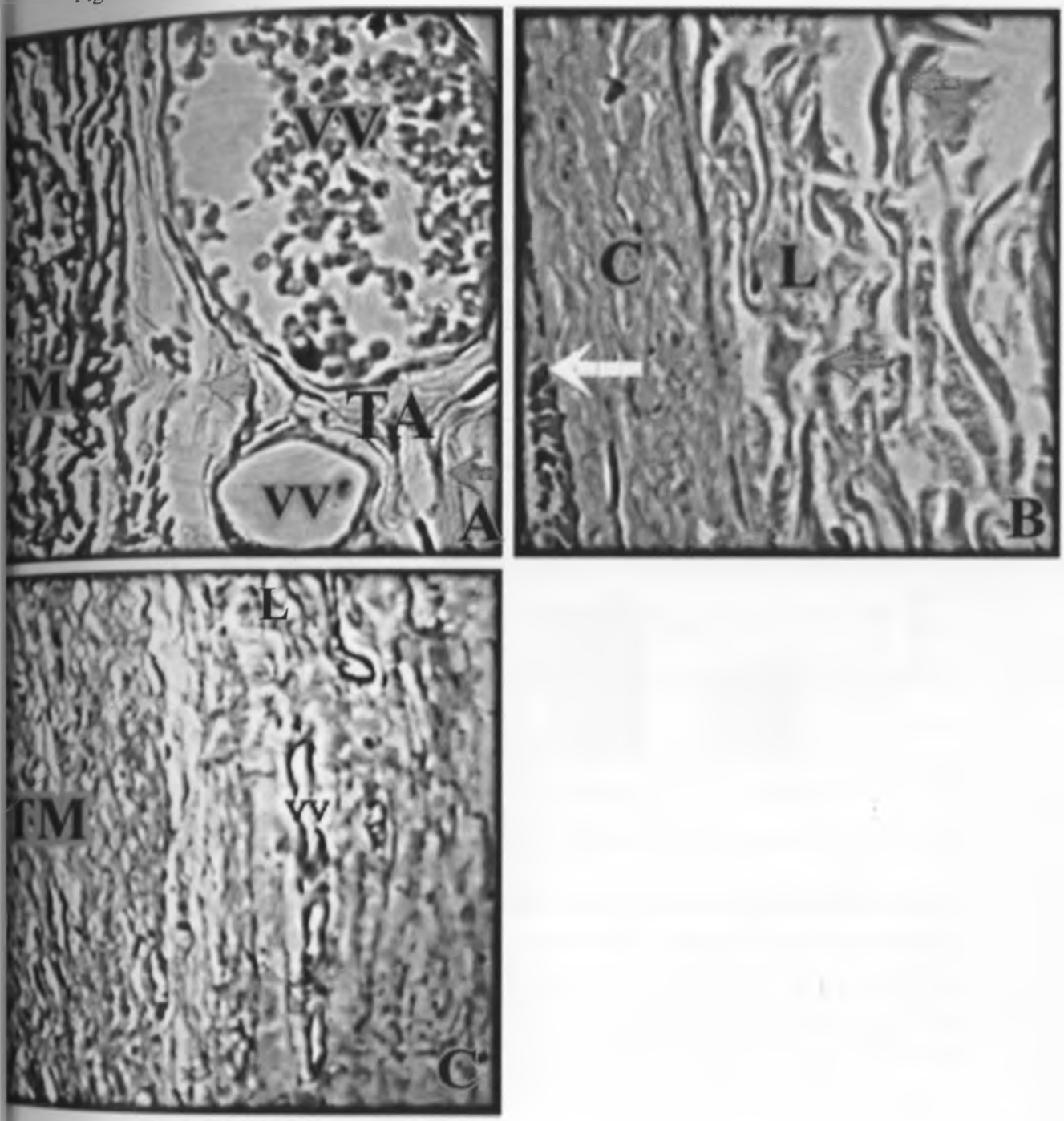
Figure 6: The adventitia of the common carotid artery

A: The tunica adventitia is predominantly fibroelastic, with interspersed prominent vesse (vv) (Magnification X400, Weigert Elastic).

B: It has two layers, the inner compact (C) and the outer loose (L) zone. The EEL (Whi arrow), and collagen (red arrow) are shown (Magnification X100, Mason Trichrome).

C: It also has prominent vasa vasora (vv) in the loose zone (L) (Magnification X100, Maso Trichrome).

Figure 6: Photomicrograph of the adventitia of the common carotid artery

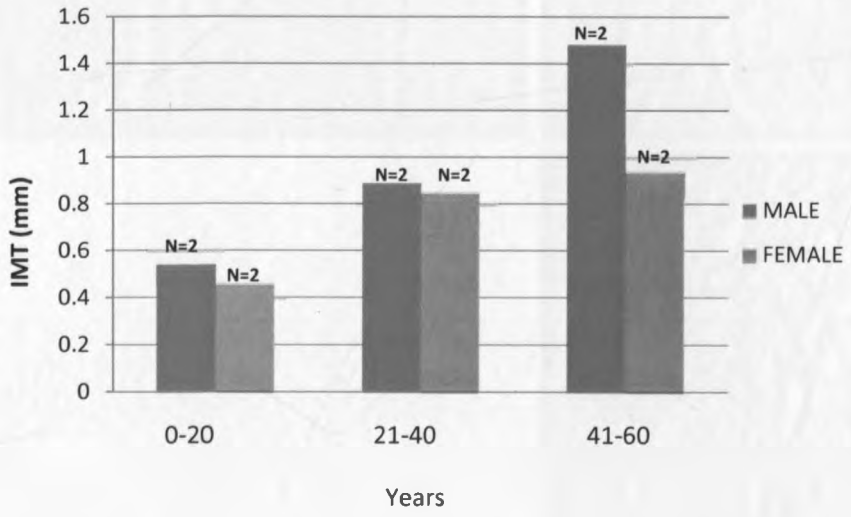


3.1 Histomorphometry of the common carotid artery

Mean carotid intimomedial thickness was 0.86±0.22 mm. The mean proximal, middle and distal carotid intimomedial thickness were 0.86±0.26 mm, 0.84±0.28mm and 0.90±0.35mm respectively (Table 1) [p=0.08]. Males (0.97±0.22) had a thicker intimomedial thickness when compared to females (0.77±0.06), [p=0.05].

Carotid intimomedial thickness increased with age; 0.5±0.16mm, 0.87±0.24mm and 1.21±0.36 mm for the age groups 0-20, 21-40 and >40 respectively [p=0.035] (Figure 7).

Figure 7: Intimomedial thickness in different age groups.



3.2 Age and gender differences in the structure of the common carotid artery

There were no obvious gender differences in the histological organization of the carotid artery. Gender differences in the arterial structure became manifest in the age group 21 to 40 years. Males had a thicker arterial wall compared to females (Table 1, Figure 10). Males had a thicker subendothelial zone when compared to females (Figure 10). The larger subendothelial zone was composed of larger smooth muscle cells and abundant collagen with some elastic fibres. Features of early atherosclerosis such as focal intimal thickening, Intimal hyperplasia and increased intimomedial thickening occurred from the 3rd decade in males and

the 4th decade in females. Males had a less prominent internal elastic lamella when compared to females. The tunica media was thicker among males as compared to females. The media in males had about 30 - 35 thick circumferential elastic fibre lamellae. In comparison, females had 27 - 34 concentric lamellae (Fig. 8). There were no gender differences in the structure of the adventitia. The males had more prominent vasa vasora when compared to females.

3.3 Regional differences in the structure of the common carotid artery

The common carotid artery showed segmental differences in the structure of the three tunics. The tunica intima in the proximal segment comprises of a single layer of endothelial cells and the SEZ and was 0.86±0.26 mm in thickness. These findings decrease distally such that in the middle it was 0.84+0.28mm while the distal point it increased to 0.90+0.35 (Table 1).

The tunica media in the proximal segment comprises of elastic lamella with interspersed collagen and SMC and was 0.77±0.31 mm (Table 1). Its size reduced relatively from proximal to distal such that it was 0.59±0.34mm in the middle segment and 0.59±0.41mm in the distal segment. The content of smooth muscle increased distally, developing an inner muscular medial layer and an outer fibro elastic medial layer. The number of elastic lamellae decreased distally from 35 in the proximal segment, 28 in the middle segment and 25 in the distal segment.

The thickness of the size of the tunica adventitia increased distally, developing from a single layered tunic to a bilayered structure distally, with an inner compact and an outer loose zone (Fig. 6, Fig. 8A-C). The diameter and number of the vasa vasora increased distally.

Figure 8: Gender differences in the tunica media

A: Photomicrograph showing the tunica media (TM) in a 25 year old female. The tunica media had 20 concentric elastic lamellae. Magnification X 40, Weigert Elastic.

B: Photomicrograph showing the tunica media (TM) in a 26 year old male. The tunica media had 30 concentric elastic lamellae. Magnification X 40, Weigert Elastic.

Figure 8: A photomicrograph showing gender differences in the tunica media

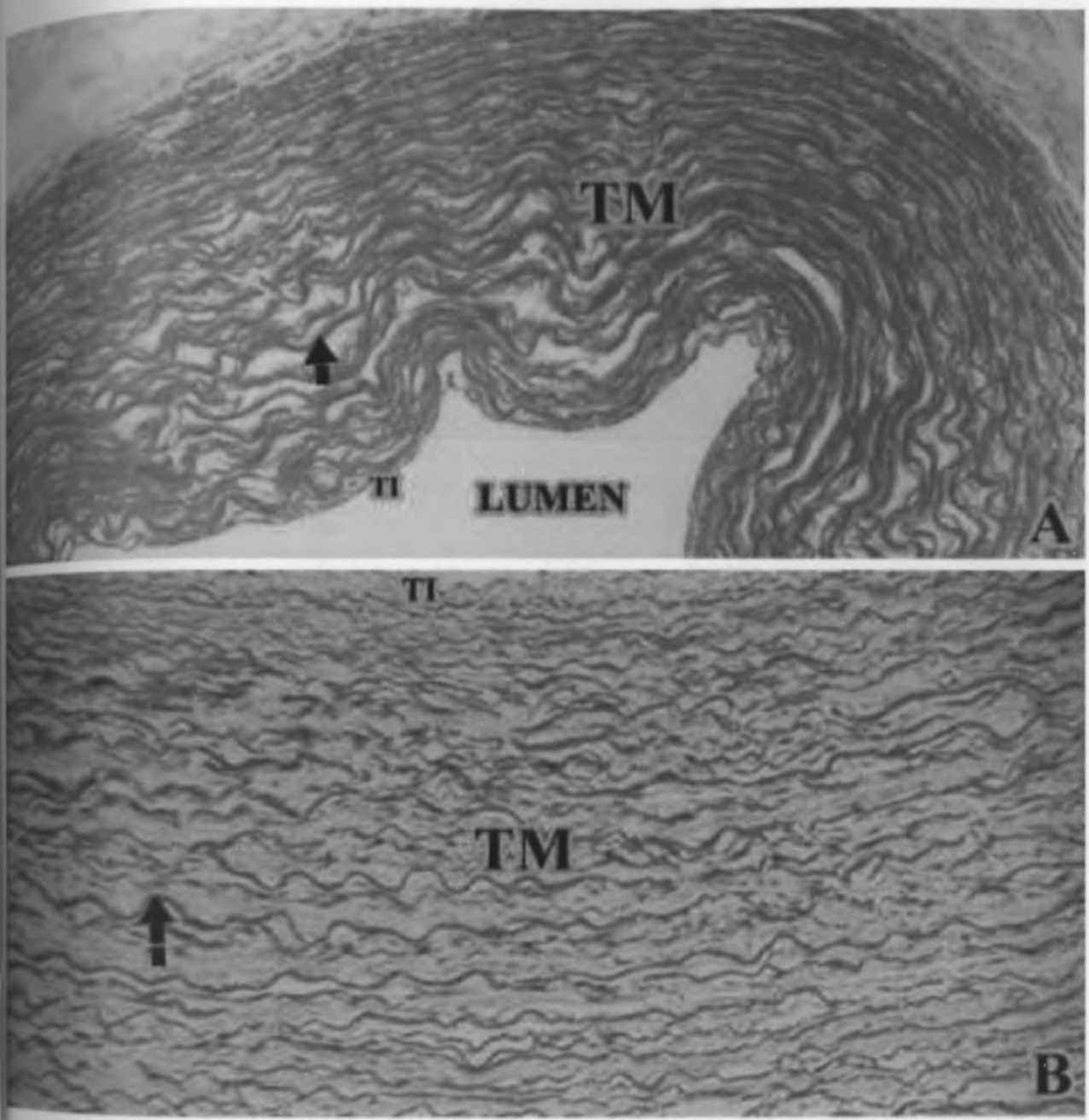


Table 1: Carotid intimomedial thickness across the segments, age and gender groups

Age group	Gender	SEGMENT		
		IMT A (mm)	IMT B (mm)	IMT C (mm)
0-10	Male (n=1)	0.64	0.47	0.52
	Female (n=1)	0.65	0.38	0.23
11-20	Male (n=1)	0.60	0.51	0.53
	Female (n=1)	0.78	0.40	0.51
21-30	Male (n=1)	0.81	0.70	0.82
	Female (n=1)	0.72	0.65	0.73
31-40	Male (n=1)	0.90	1.00	1.16
	Female (n=1)	0.89	1.03	0.89
41-50	Male (n=1)	1.33	1.32	1.34
	Female (n=1)	0.68	0.90	1.0
51-60	Male (n=1)	1.39	1.50	2.00
	Female (n=1)	0.72	1.15	1.20

3.4 Androgen receptor distribution in the common carotid artery

Androgen receptors were not detected in all the segments, age and gender groups of the common carotid artery (Table 2; Figure 11 A-F).

Table 2: Distribution of androgen receptors in the common carotid artery

AGE GROUP		SECTION		
		Proximal	Middle	Distal
A	TCC	27	25	29
	AR+	0	0	0
B	TCC	28	26	30
	AR+	0	0	0
C	TCC	29	29	32
	AR+	0	0	0

TCC – Total cell count per field.
AR+ - Total number of immunopositive cells for AR.

Figure 9: Regional differences in the structure of the tunica adventitia

A: Photomicrograph of a proximal section of the CCA showing the tunica Adventitia (TA) and Tunica Media (TM), separated by an external elastic lamella (Arrowed). (Magnification x100, MT stain).

B: Photomicrograph of a middle section of the CCA showing the tunica Adventitia (TA) and Tunica Media (TM), separated by an external elastic lamella (Arrowed). The tunica adventitia has two layers compact (C) and loose (L). (Magnification x100, MT stain).

C: Photomicrograph of a distal section of the CCA showing the tunica Adventitia (TA) and Tunica Media (TM). The tunica adventitia has two layers compact (C) and loose (L). (Magnification x100, MT stain).

Figure 9: A photomicrograph showing regional differences in the structure of the tunica adventitia

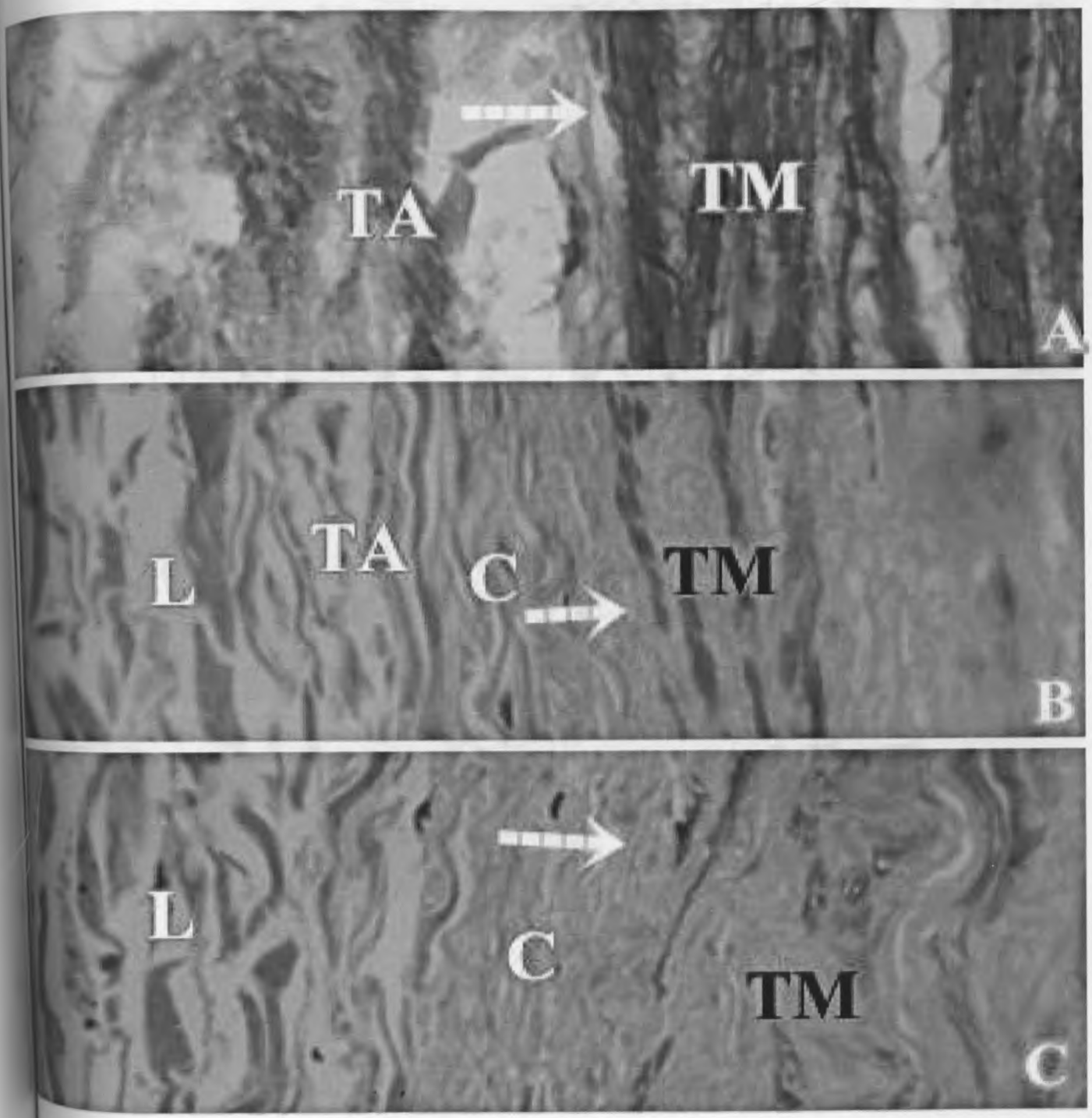


Figure 10: Age and gender differences the carotid tunica intima

A: Photomicrograph of the tunica intima of the proximal segment of the CCA in a 12 year old female. The endothelial cells are flat and short. The SEZ is composed of some SMCs, abundant collagen fibers and elastic fibers. The size of smooth muscle cells is relatively small (Magnification X100, Masson Trichrome).

B: Photomicrograph of the tunica intima of the proximal segment of the CCA in a 30 year old male. Endothelial cells are larger and elongated. The SEZ is composed of more SMCs with relatively reduced collagen fibers. (Magnification x100, Masson trichrome).

C: Photomicrograph of the tunica intima of the proximal segment of the CCA in a 60 year old female. The SEZ is composed of large SMC between collagen and elastic lamellae. VV are noted in this section (Magnification X100, Masson trichrome).

Figure 10: A photomicrograph showing the age and gender differences the carotid tunica intima

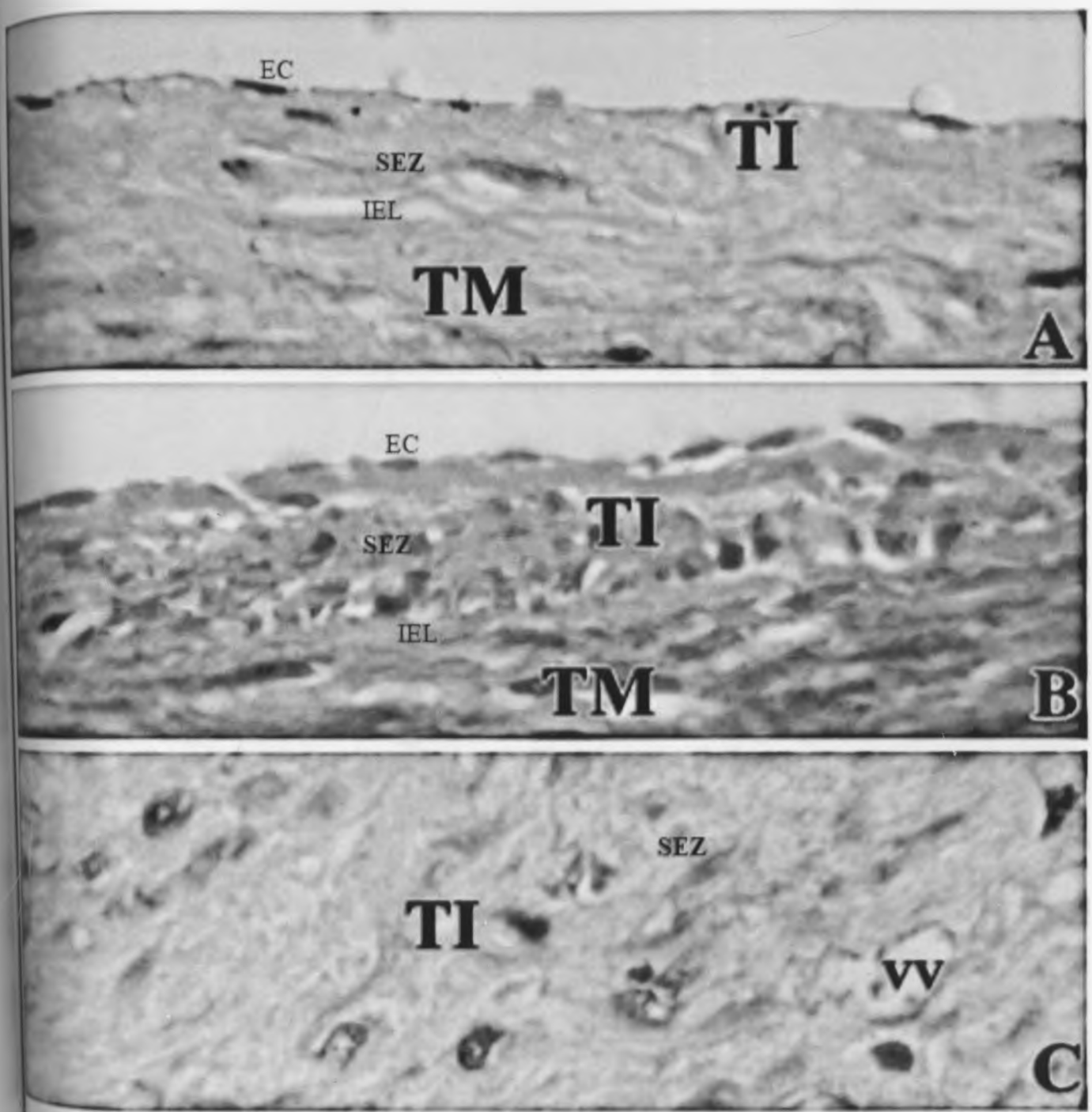


Figure 11: Non expression of androgen receptors in the carotid arterial wall

A: Subendothelial zone of the proximal segment of the carotid artery in a 25 year old female. Smooth muscle cell nuclei without androgen receptors are stained (Arrowed white).

B: The carotid artery tunica media in a 12 year old female is shown. There was no positive staining of androgen receptors in the smooth muscle cells arrowed white.

C: The common carotid artery tunica media in a 30 year old male is shown. Androgen receptors are not expressed in the smooth muscle cells (White arrow).

D: A positive control (post-mortem prostate) expressing brown stained nuclei with androgen receptors (Black arrow).

E: A positive control (post-mortem prostate) expressing brown stained nuclei with androgen receptors (AR- 318 positive cells black Arrow).

F: A negative control did not stain any androgen receptors (White arrow).

Magnification X 400 for figures A-F

Figure 11: A photomicrograph showing the non-expression of androgen receptors in the carotid arterial wall

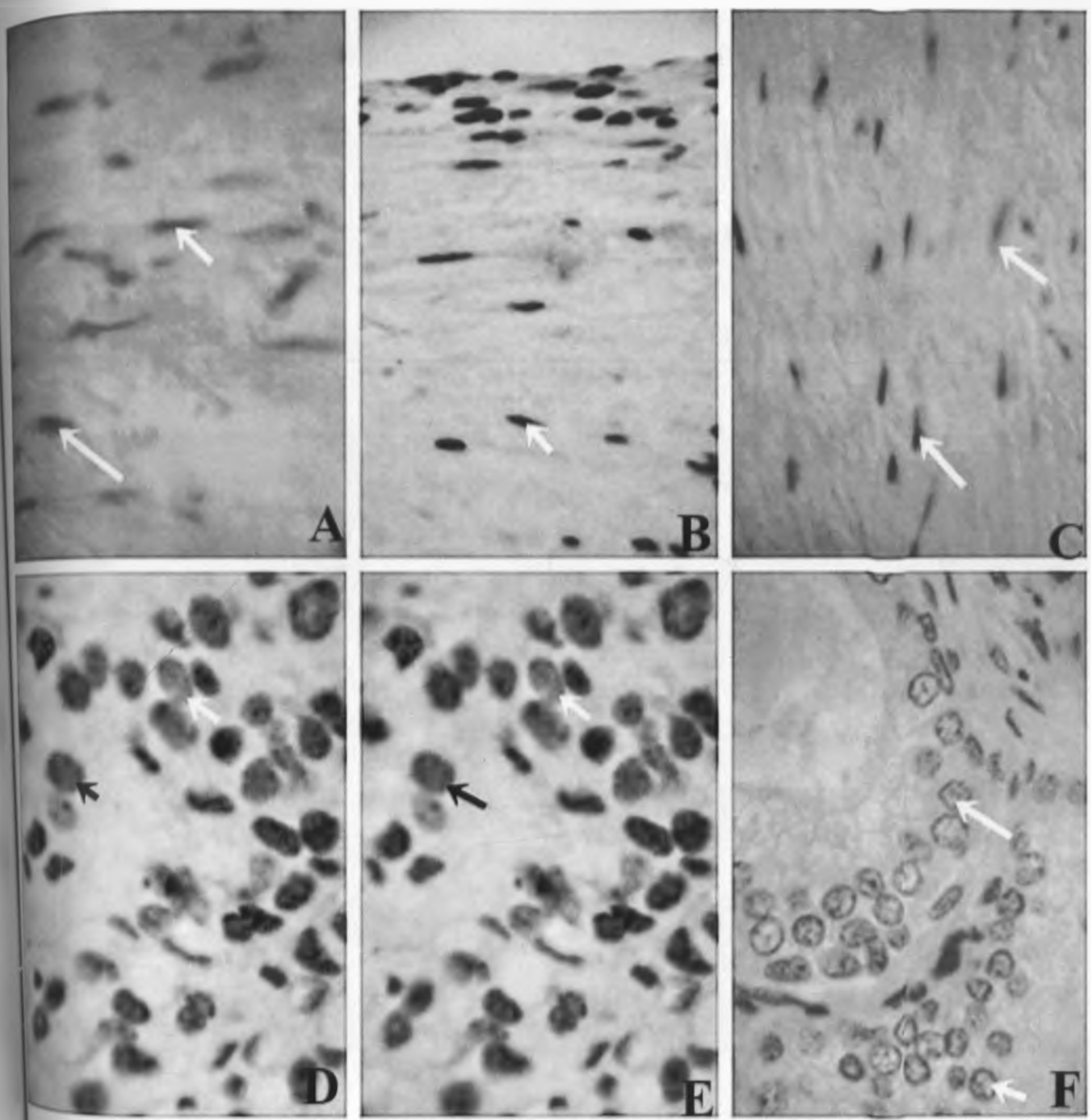


Table 3: Carotid Intimomedial thickness in various populations

Study	Population	Method	IMT (mm)
Crouse et al., 1995	US	ultrasound	1.049
Simons et al., 1999	Caucasian	Ultrasound	0.94
Okeahialam et al., 2011	Nigeria	Ultrasound	0.91
Bots et al., 2005	Netherlands		0.82
Adaikkappan et al., 2002	Indian	Ultrasound	0.73
Özdemir et al., 2006	Turkey	Ultrasound	0.62
Sharma et al., 2009	Nepalese	Ultrasound	0.55
Pignoli et al., 1986	Italian	Histology	0.48
Present study	Kenya	Histology	0.86

Table 4: Age differences in the carotid intimomedial thickness

Study	Population	IMT (mm)		
		0-20	21-40	41-60
Ludwig et al., 2006	US	0.5	-	0.9
Ma et al., 2011	Taiwan			0.77
Pignoli et al., 1986	Italian		0.48	
Present study	Kenya	0.77	0.87	1.21

Table 5: Gender differences in the carotid intimomedial thickness

Study	Population	IMT (mm)	
		Males	Females
Crouse et al., 1996	US	0.83	0.76
Ziembicka et al., 2005	Poland	1.05	0.93
Lawlor et al., 2004	British	1.54	1.36
Ma et al., 2011	Taiwan	0.77	0.72
Present study	Kenya	0.97	0.77

CHAPTER FOUR: DISCUSSION AND CONCLUSIONS

4.1 **Histomorphology of the Human Common Carotid Artery**

The common carotid artery comprises of three histological layers which had also been described in other arteries. The tunica intima comprises of a single layer of flat endothelial cells and the sub endothelial zone, consistent with reports from sheep, goats, giraffe and dogs (Kimani, 1983; Orsi et al., 2006; Parchami et al. 2009). Flat endothelial cells have better adaptation to the shear stress of a conduit vessel like the common carotid artery (Fisher et al., 2001; Fisher et al., 2002). In concurrence with observations made in other mammals, the sub endothelial zone was composed of collagen, elastic fibres and smooth muscle cells in different proportions (Kimani, 1981; Parke et al., 1995; Parchami et al., 2009). Sub endothelial smooth muscle cells were arranged axially as observed in the bovine CCA and pig aorta (Stergiopulos et al., 2001; Timmins et al., 2010). The axial orientation of subendothelial vascular smooth muscle cells is useful in axial loading of pressure from the endothelial cells to the inner medial layer (Clark and Glagov, 1985). Subendothelial smooth muscle cells synthesise extracellular matrix and are prone to fatty infiltration (Schwartz et al., 1986; Aidinian et al., 2006). The subendothelial layer supports loads that are in the axial direction (Clark and Glagov, 1985), and is prone to remodeling related to the stresses exposed to it (Kimani, 1983) leading to thickening.

The CCA intima displayed focal thickening and intimal hyperplasia. This thickened intima was composed of large smooth muscle cells, collagen and elastic tissue, consistent with previous observations (Virmani et al., 2000). Intimal thickening is a reservoir for lipid retention and is a surrogate marker of atherosclerosis (Wilens, 1951; Nakashima et al., 2007). The observed wavy internal elastic lamella allows for distension of the artery and acts as a

macromolecular barrier between the two layers (Penn et al., 1994; Laurent et al., 2005). In some sections, and areas of intimal hyperplasia, the IEL was split into two. This feature has been seen in muscular arteries and in pathological states such as hypertension and intimal cushions (Todd and Friedman, 1972; Yamazoe et al., 1990). The wavy folding in the IEL observed in CCA is attributed to subendothelial smooth muscle insertion (Krsti, 2009).

The tunica media was the widest zone of the carotid artery wall. It was predominantly elastic as seen in dogs and rats (Pinto et al., 1998; Orsi et al., 2006). This layer is muscular in goats, sheep and giraffe (Kimani, 1983; Parchami et al., 2009). Elastic fibers ensure uniform tension distribution throughout vessel walls and are primarily responsible for the elastic recoil properties of large elastic arteries (Wagenseil et al., 2005). Elastic recoil imparts a hydraulic advantage to the circulatory system by converting intermittent cardiac output into steady flow, thereby reducing cardiac workload (Shadwick, 1999; Faury, 2001; Safar et al., 2003).

The tunica media was composed of 30-35 concentric elastic lamellae, more than the nine seen in rats (Pinto et al., 1998) and fifteen reported in dogs (Orsi et al., 2006). The number of elastic lamellae in the tunica media is related to the size of the mammal (Wolinsky and Glagov, 1967). Elastic lamellae were oriented circumferentially to allow for vascular distension of this conduit artery (Timmins et al., 2010). The propensity of the carotid artery to develop atherosclerosis is not to be anchored in the elastic nature of its tunica media because muscular arteries such as the coronaries are also predisposed to atherosclerosis (Hansson, 2005). It has however been noted that a decline in the amount and quality of elastic tissue in the tunica media makes elastic arteries unable to tightly set stress force values thus creating local wall shear stress reductions that predispose individuals to intimal thickening,

which also contributes to vascular tensile support (Masawa et al., 1994; Caralo et al., 1999). In support of the observations made by some authors, medial smooth muscle cells were oriented circumferentially (Wolinsky and Glagov, 1967; Arner and Uvelius, 1982; Dingemans et al., 2000), contrary to the observations made by Bierring and Kobayashi, who observed oblique orientation of medial SMC (Bierring and Kobayashi, 1963). In some sections, a thin subintimal smooth muscle zone was observed. Subintimal smooth muscle cells are early features of atherosclerosis (Hartman, 1977). The presence of these cells explains why the carotid artery is at risk of atherosclerosis.

The tunica adventitia of the carotid arteries was composed of elastic fibers, collagen fibres with longitudinal, transverse and oblique arrangements. This composition and arrangement of fibers is also observed in femoral and renal arteries (Gutterman, 1999). In middle and distal sections, the tunica adventitia had two zones, inner compact and outer loose. There are no previous reports on the zonation of the tunica adventitia. The compact inner layer of the adventitia may offer more support to the tunica media, when compared to the generally loose tunica media especially in elevated blood pressure. Also, the adventitial elastic fibres and lamellae showed a variable pattern, with a predominance of circumferential dispositions. This arrangement was similarly reported for the CCA of the dog (Orsi et al. 2006). The different spatial arrangements of the connective fibres observed in the adventitial layer perhaps help to guarantee the vascular wall integrity and protect the arterial wall.

The human CCA had prominent vasa vasora extending into the tunica media and intima. This was consistent with the increase in the thickness of the vessel wall from 0.5mm to 1.21mm in the young and the elderly respectively. Bo et al (1989) observed that when the thickness of an artery exceeds the ability of simple diffusion of nutrients from the lumen, vasa vasora extend

to supply the media and intima. This extensive network of vasa vasora in the adventitia arises from branch points of parent arteries. Vasa vasora in the media and intima arise predominantly from adventitial vasa, but can arise from the lumen (Williams and Heistad, 1996; Bayer et al., 2002). The large size of the CCA vasa vasora is of clinical significance as they are related to the size of the vessel wall (Gossl et al., 2003) and are a marker of vessel remodelling to meet its metabolic needs.

4.2 Histomorphometry of the Common carotid artery

The carotid intimomedial thickness among Kenyans of 0.86mm found by histology closely compares with ultrasound results observed in Netherlands and Nigerians [Table 3] (Bots et al., 2005; Okealahim et al., 2011). Although there is a difference in the methods used, intimomedial thickness measured by ultrasound correlates significantly with the intimomedial thickness determined by histology (Pignoli et al., 1986; Choi et al., 2009). The difference between the intimal medial thickness in the present sample and the 0.48mm observed by Pignoli et al (1986) on microscopy is remarkable. Although there were similarities in sample preparation, differences exist in the sample size, segment, age and measurement method used. Pignoli evaluated 44 male as opposed to the 12 (6 male, 6 female) carotid samples used in the present study. Their samples aged between 20-25yrs while our samples ranged from 1-60 yrs. When adjusted for age and gender, carotid intimal medial thickness in samples between 21-30yrs in the present study were 0.74, still higher than the observations of Pignoli. Furthermore the CCA segments measured by Pignoli were unclear. Finally, Pignoli et al (1986) used a graduated ocular piece for measurement, while the present study used the Scion Image™ Multiscan software for intimal medial thickness assessment. It is however unclear as

to whether methodology, ethnic, or lifestyle differences could explain this remarkable difference in IMT.

This thickness was lower than 1.049mm reported in Americans and it was higher than 0.73mm reported in Indians (Crouse et al., 1995; Adaikkappan et al., 2002). Intimomedial thickness in Kenyans was also lower than the 0.94mm reported in Caucasians, in contrast with earlier reports that IMT is significantly higher in blacks than Caucasians (DÁgostino et al., 1996; Chambless et al., 1997; Urbina et al., 2002). Carotid intimomedial thickness shows population related differences, which are to be attributed to the distribution of cardiovascular risk factors in these populations. The IMT of the CCA is a good marker for both the presence of early arteriosclerosis (Pignoli et al., 1986; Grobbee and Bots, 1994) and the degree of arteriosclerosis of an individual (Ebrahim et al., 1999; Simons et al., 1999; Frauchiger et al., 2001). Increases in the thickness of the tunica intima and media of the carotid artery are directly associated with an increased risk of atherosclerosis in other vascular beds such as the coronary arteries (Riley et al., 1986; Arnet et al., 1994; Salonen and Salonen, 1991; O'Leary et al., 1999). The critical measure of carotid intimomedial thickness which imperatively predicts atherosclerosis is however still unclear, even in the present study. The utility of the current measure in a Kenyan population is limited by tissue shrinkage, sample size and selection challenges, which affected the measured intimomedial thickness. These pilot observations in the Kenyan sample can be anchored by follow-up in vivo studies using doppler ultrasound.

An increased CIMT may reflect a nonatherosclerotic adaptive arterial response (Bots et al., 1997a; Glagov et al., 1987). Common carotid IMT is related to changes in local shear stress and tensile stress (Bots et al., 1997b). It has also been reported that increased IMT and atherosclerotic plaques are overcompensated by an accompanied increase in lumen diameter

of the common carotid arteries (Bonithon-Kopp et al., 1996). When arterial enlargement accompanies increased wall thickness, less lumen constriction results than expected (Crouse et al., 1994; Crouse et al., 1996).

4.3 Segmental differences in the histomorphometry of the common carotid artery

Observations of the present study reveal that there are segmental changes in the structure of the carotid artery. These include increase in the intimomedial thickness; reduction in the number of elastic lamellae, increase in the amount of tunica media vascular smooth muscle cells, development of two layers of the tunica adventitia and increase in size of the vasa vasora along the vessel profile. The increase in the thickness of the intimomedial complex along the vessel profile observed in the CCA could be theoretically related to the sliding of the mural coats and to the local hemodynamic stress on the vascular wall layers at the origin of the CCA and towards the carotid bifurcation (Kimani, 1983; Milner et al., 1998; Willekes et al., 1999). Observations of the current study imply that the distal segment of the carotid artery is prone to atherosclerosis as compared to the middle or proximal segment. These observations also suggest that the distal carotid artery is more adapted to the hemodynamic stresses exposed to it (Kamiya and Togawa 1980; Glagov, 1994). Nevertheless, distal thickening of the carotid intimomedial thickness occurs earlier than any other segment of the artery (Persson et al., 1994). This observation also explain the conclusions by Ebrahim et al (1999), that IMT measurements in vivo should focus on the distal segment of the carotid artery to give a more clinically useful parameter. The reduction of the number of elastic lamellae with concurrent increase in the amount of smooth muscle cells in the tunica media along the vessel profile is to be attributed to the gradual transition of the arterial structure from elastic to muscular. This transition is however completed in the more distal branches of the carotid artery (Schievink et al., 1994).

4.4 Age and gender differences in structure and morphometry of the common carotid artery

The carotid artery displayed age and gender difference in its dimensions.

4.4.1: Age differences in structure and morphometry of the common carotid artery

Carotid intimomedial thickness increased with age, supporting observations made in previous studies [Table 4] (Hort et al., 1982; Labropoulos et al., 1998; Osika et al., 2009; Cobble and Bale, 2010; Jarauta et al., 2010). The increased intimomedial thickness comprised more elastic lamellae, smooth muscle cells and size, and increased collagen deposition. Age changes in the structure of the carotid artery including diffuse intimal thickening has been reported to develop from an early age in human arteries before atherosclerosis evolves (Movat et al., 1958; Nakashima et al., 2002; Nilsson et al., 2008). Changes in the structure of the carotid artery with age could be attributed to hemodynamic differences related to the variability of the carotid bifurcation with age (Goubergrits et al., 2002), as well as lifestyle changes (Okada et al., 2004). Individuals from adolescent age should be encouraged to adopt cardiovascular friendly lifestyles to slow the effects of aging on the carotid artery which would otherwise complicate into atherosclerosis later in life.

4.4.2: Gender differences in structure and morphometry of the common carotid artery

Carotid intimomedial thickness was thicker in males than females, consistent with previous reports in literature [Table 5] (Crouse et al., 1996; Ebrahim et al., 1999) that IMT shows gender dimorphism. The observed wider intimomedial thickness in males begins from adolescence and partly explains why males are more prone to atherosclerosis when compared to females (Bohm et al., 2009). Contrary to previous observations (Sinning et al., 2011), the

gender differences in the dimensions of the carotid intima and media were pronounced in the third age group especially among males. Males in the third age group had unusually thick vessel walls compared to the females. This disparity could be in part related to the differences in the ethnic groups and age groups considered and our small sample size. Nonetheless postmenopausal had increased intimomedial thickness, significantly more than their premenopausal counterparts. The age difference in the intimomedial thickness between the females in the third age group and the females in the second age group was comparable to the gender difference in the CIMT. This suggests that menopause is accompanied with significant increase in carotid intimomedial thickness in support of the observations made by Espeland et al (1995). The similarity in pattern of difference in the intimomedial thickness between the genders, and between the post-menopausal premenopausal females generally also implies that females are masculinized after menopause. The gender difference in the structure of the vessel seems to be related to the effects of oestrogens and androgens.

4.5 Androgen receptor expression in the common carotid artery

In the present study, androgen receptors were not expressed in any of the layers, segments or age groups of the common carotid artery. Findings of the present study are at variance with the observations of Liu et al (2005) who immunolocalised androgen receptors in the post-mortem coronary arterial wall, inversely relating their numbers to early atherosclerosis. The absence of these receptors in the carotid wall suggests that androgen receptors have a limited genomic role in the carotid arterial structure and subsequent development of atherosclerosis. A similar conclusion was made by Christian et al., (2006), who did not localise AR in the coronary arterial wall. On the contrary, McRobb et al (2009) immunolocalised AR in the rat innominate artery and the aortic sinus and positively associated them with increased

calcification of the atherosclerotic plaques. While androgens play a role in the cardiovascular system, the present study supports the observations that some androgen effects (non-genomic) in arteries are not mediated through androgen receptors (Chou et al., 1996; Costarella et al., 1996; Rubio et al., 1998; Reckelhoff et al., 1999; Williams et al., 2002). In addition, the present observations suggest that the perceived gender disparity in the structure of the carotid artery with worsening atherosclerosis is largely unrelated to the effects of androgens. Since androgen receptors were not localised in the common carotid arterial wall, it is has therefore likely that the view that androgens are solely 'harmful' or 'beneficial' is simplistic as regards the understanding of the effects of androgen receptors on atherosclerosis. Findings of the present study also failed to link androgen receptor expression to the perceived gender dichotomy in the structure of the carotid artery as previously suggested (Parchami and Dehkordi, 2011). Despite this, gender disparity in cardiovascular disease persists with androgen use and abuse is increasing in our population, either for therapeutic or recreational reasons. Whether androgens adversely affect CVD in either men or women remains a contentious issue that is in desperate need of more research.

The absence of the AR in the CCA could also be partly attributed to post-mortem loss or low numbers limiting detection (Mainwaring and Mangan, 1973), since Fodor et al (2002) found that the post-mortem stay before fixation and the duration formalin fixation affects the expression of steroid receptors in post-mortem material. Steroid receptors deteriorate gradually after 24hrs before fixation and their retrieval is reduced after 20 days of formalin fixation. In this regard, there should have been residual cells stained even if most of them were hydrolysed post-mortem. In addition, the prostate controls which were harvested at the same post-mortem duration expressed the receptors, supporting the thought that these receptors were in few numbers that could not be detected. Fodor et al (2002) also observed

that microwave retrieval androgen receptor epitopes from formalin fixed samples occurs even after 20 days of fixation. This suggests that the receptors should have been extracted if they were present in the artery. Lastly, the vascular androgen receptors could have different epitopes when compared to prostatic carcinoma ones. This is supported by the observed heterogeneity of the androgen receptors expressed in prostatic cancer tissue (De winter et al., 2005). Monoclonal (MAb) androgen receptor marker used in the present study may not suitably stain the carotid androgen receptors because a small change in the structure of an epitope (e.g., as a consequence of genetic polymorphism, glycosylation, and sumoylation) can markedly affect the function of a MAb (Thomas et al., 2004; Lipman et al., 2005) in staining the vascular AR, leading to a false absence of this receptors.

Conclusions

The human common carotid is an elastic artery and its structure shows regional and age differences in dimensions. The distal carotid has the largest intimomedial thickness. The carotid artery does not display gender differences in the histological organization although males have a thicker intimomedial thickness than females. Postmenopausal women have a masculinized CIMT. There was no immunostaining of AR-318 in the carotid specimens. Histological features of early atherosclerosis are manifest from the 3rd decade in males and 4th decade in female.

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APPENDIX 1

CONSENT FORM.

Study number: _____

This form will be used to give a brief summary of the present study to the relatives of the deceased. After the information, relatives who consent for the sections of the carotid artery of the deceased to be used in this study will sign the present form as an approval for the use of the deceased material in the present study.

Background: We are carrying out a survey on the carotid artery intimomedial thickness and androgen receptors. We shall use cadaveric material for this study.

Aim of the study: This study will be assessing the carotid artery intimomedial thickness and androgen receptors in man.

Benefits: Information obtained from this study may help to underscore the anatomy of the carotid arteries among Kenyans. This will guide clinicians when approaching this area which is delicate in surgery among Kenyans.

Confidentiality: The name of the deceased shall not be disclosed or used in the study.

Extent of harvesting: Only three 5 mm section of the vessel wall shall be harvested for the present study.

Disposal: Material that may be useful in the subsequent related study shall be retained. All other material harvested shall be buried at Langata Cemetary after the present study.

I, the undersigned, having been explained to and understood the importance of this study, do allow the cadaver to be enrolled in the study.

_____

Name and title Signature

We, the investigators, having explained in detail the purpose of the study, hereby submit that privacy shall be maintained and only details related to this study will be published.

_____

_____

Signature Date

Contact of the principal investigator: Tel 0721486182

Contact of KNH/UON-ERC Chairman: 0202726300 ext 44102

CHETI CHA RUHUSA

Nambari ya uchunguzi: _____

Fomu hii itatumiwa kuwajulisha jamii ya aliyefariki kuhusu uchunguzi ambao tunanuia kufanya. Baada ya maelezo, ikiwa jamii watakubali, watatia sahii kenye fomu hii kutoa idhini kwa wachunguzi kutumia sehemu ya mishipa ya alieyafriki kwa uchunguzi huu.

Kitambulisho: Sisi tunanuia kufanya uchunguzi wa mshipa ya damu ya shingo uitwao carotid kwa watu waliofariki.

Lengo la uchunguzi: Uchunguzi huu utatuwezesha kutambua maumbile ya mshipa huu haswa katika wakenya kwani umeonyesha mabadiliko mbali mbali.

Manufaa: Uchunguzi huu utasaidia madaktari wa upasuaji na madaktari wengine kufanya upasuaji salama haswa katika wakenya wenye maumbile tunayo ya chambua.

Siri: Aliyafariki hatatambuliwa kwa jina lake katika uchunguzi huu.

Kiwango cha uchunguzi: Sehemu ndogo ya mshipa wake zenye urefu wa milimita tano pekee itachikuliwa katika uchunguzi huu.

Mazishi: Sehemu zote zilizo chukuliwa zitazikwa Lang'ata cemetery baada ya uchunguzi huu.

Mimi, nadhibitisha kwamba nimeelezwa na nikafahamu lengo na manufaa ya uchunguzi huu na kwa hiari yangu mwenyewe kuwapa wachunguzi idhini ya kuhusisha mwili wa marehemu katika uchunguzi huu.

_____

Jina na Hadhi Sahihi

Sisi watafiti tunadhibitisha kwamba tumemuelezea mlezi kuhusu uchunguzi huu ipasavyo na kwamba habari inayohusiana na uchunguzi huu tu ndio utakao chapishwa.

_____

_____

Sahihi Tarehe

Nambari ya simu ya: mtafiti mkuu: tel 0721486182

Mwenyekiti wa kamati chunguzi: 020 2726300 ext 44102

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