Atherosclerotic lesions in the thoracic aorta: A South African anatomical and histological mortuary study

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Background. Worldwide, the prevalence of cardiovascular diseases such as atherosclerosis is on the increase. Younger people may be especially vulnerable owing to their exposure to risk factors such as drug abuse and HIV.

Methods. The thoracic aortas of 149 South Africans under the age of 50 years were collected at the Salt River Mortuary, Cape Town, and examined macroscopically and microscopically for evidence of anomalies. The sample comprised predominantly males, and included black, coloured and white individuals.

Results. A significantly higher level of macroscopic pathology was found in coloured males, although overall prevalence of pathology in this sample was lower than expected. A positive association was also found between body mass index and vascular pathology in the black and coloured population groups. Microscopic anomalies were common and present at high levels, irrespective of age and racial grouping.

Conclusions. The widespread prevalence of microscopic anomalies in all groups suggests that these are normal variations that result from haemodynamic forces. The higher prevalence of atherosclerotic lesions in coloured males, however, probably results from specific genetic conditions such as hypercholesterolaemia or lifestyle factors such as diet or tik abuse. The findings suggest that coloured individuals may be at increased risk of developing cardiovascular disease.

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Diseases of the cardiovascular system are predicted to be the leading worldwide cause of death by 2030.[1,2] Cardiovascular diseases (CVDs) are characterised as 'diseases of lifestyle', associated with a 'Western' diet and low levels of physical activity. However, ischaemic heart disease and other non-communicable diseases are likely to be major contributors to mortality in sub-Saharan Africa.[2]

Forms of these diseases (once associated with ageing[3]) are common in younger individuals; atherosclerotic lesions, aneurysms and strokes have been reported in children and young adults.[4-6] Fatty 'dots' and streaks, which may represent the early stages of atherosclerosis, have been found in the aortas of babies and infants.[6]

Risk factors in the increasing prevalence of CVD include cigarette smoking, high-fat diet, high cholesterol levels, obesity, diabetes, hypertension and HIV infection.[1,4,7] Antiretroviral treatment for HIV-positive individuals also increases the risk of developing CVD.[8]

Certain communities in South Africa have a high potential for exposure to one or more of these recognised risk factors, as well as having a genetic predisposition to hypercholesterolaemia.[9] With the high rate of HIV infection and frequently limited access to health care, South Africans may be at increased risk of developing CVD.

South African studies indicated that early lesions such as fatty streaks were common in black South Africans, but levels of more advanced lesions were lower than those reported for white South Africans, black and white Americans, and European populations.[10,11] It has been suggested that this could be due to lower dietary fat intake and lower serum cholesterol levels in black South Africans.

In an autopsy study on atherosclerotic disease in Cape Town, it was found that black and coloured individuals had high levels of moderate to severe atherosclerotic lesions, and that advanced lesions were most common in those younger than 50 years.[12] Although our study suggested that the distribution pattern of atherosclerosis is undergoing transition in South Africa, only 58 individuals aged between 14 and 95 years were examined; and of these, 11 were older than 50 years, and only 2 were younger than 20 years.

To determine the prevalence and distribution of arterial disease in young South Africans, we studied the gross and histological appearance of the aortas of individuals under the age of 50 years.

Materials

Permission for collection of tissue samples was granted by the Health Sciences Faculty Human Research Ethics Committee (REC REF: 227/2005). For comparative purposes, only sections of the thoracic aorta were collected.

Samples of macroscopically normal and diseased thoracic aortas were obtained at autopsy from 149 randomly selected individuals at the Salt River Mortuary in Cape Town. Autopsies were performed by pathologists from the Division of Forensic Medicine and Toxicology, University of Cape Town. These individuals had died of unnatural causes, including stabbing, gunshot, assault and motor vehicle accidents. None was known to have died from cardiovascular disease or following hospitalisation for illness.

Of the 149 individuals, 138 (92%) were male and 11 (8%) were female; ages ranged from 1 month to 47 years. The race categories used in this study are those designated by the South African Police Services: black refers to indigenous Africans; white to individuals of European ancestry; and coloured to individuals of mixed (Khoi San, Indian, Indonesian, black African and European) ancestry. The sample comprised 115 (77%) black, 31 (21%) coloured, 3 (2%) white, and a single Asian (Chinese) individual. The height, weight, age and cause of death of the individuals were recorded, where available. At autopsy, each individual was allocated a number that corresponded to the Forensic Pathology Services case file number that subsequently identified specimens and histological samples.

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Methods

The attending pathologist assessed macroscopic appearance of the vessel at autopsy. A macroscopically 'normal' aorta was defined as one in which the intimal surface of the vessel was smooth, glossy and of uniform colour, with no fatty streaks or other lesions. An abnormal or diseased vessel had lesions visible to the naked eye.

Segments measuring 2 - 3 cm in length were removed from the aorta, between the aortic arch and the diaphragm. Where the aorta appeared normal, random segments were taken. In cases of macroscopic pathology, segments included the sites of lesions. The segments were placed in jars containing 10% neutral buffered formalin and labelled with the case file number and date of collection.

Each segment was then divided further into 3 sections for histological processing, 2 of which were processed and embedded into a single wax block. The third section was dried and embedded into Tissue Freezing Medium (Leica Microsystems, Nussloch) to determine the presence of lipids. Sections with a thickness of 5µm were obtained from each wax block on a Reichert-Jung Autocut 2040 microtome and were processed further using 3 different staining techniques: haematoxylin and eosin (H&E) to assess general vessel architecture; alcian blue pH 2.5-Periodic Schiff reactions (AB-PAS), for presence of neutral and acid mucopolysaccharides in the arterial wall; and Elastin von Gieson's (EVG) for morphology of elastic tissue, collagen fibres and smooth muscle throughout the vessel. Sections embedded in Tissue Freezing Medium were sectioned on a Leica CM 1850 cryostat, fixed on APTESE (Merck, Germany)-coated slides, and allowed to air-dry. Slides were then stained with Oil Red O (ORO).

All stained slides were viewed by light microscopy, and the general features of the vessel were observed and recorded.

Data analysis

Samples of the aorta in which no macroscopic pathology was observed were grouped as 'Normal', and those with evidence of gross pathology as 'Pathology'. Histologically, a 'Normal' aorta was characterised as having a tunica intima that was relatively thin and loose, with regularly arranged smooth-muscle cells and elastin fibres in the tunica media, and a loosely organised tunica adventitia. 'Pathology' comprised two subgroups: (*i*) samples with cells not normally seen in significant numbers in the vessel wall, or cells that indicate disease or inflammatory processes, e.g. lymphocytes and foamy macrophages; and (*ii*) samples that had alterations to normal vessel architecture or morphology, e.g. disturbances in the smooth muscle, collagen or elastin fibres. To verify microscopic observations and reduce observer error, cellular pathologies were confirmed by a pathologist on a random sample of slides. All slides were then viewed and verified simultaneously by 3 participating observers.

For each of the macroscopic and microscopic categories of 'Normal' and 'Pathology', data were analysed regarding race, age and body mass index (BMI). Fisher's exact test[22] was applied to determine statistical significance ($p<0.05$), using Statistica (Starsoft, Tulsa, USA). There were too few female samples ($N=11$) for valid statistical comparisons between males and females. Individuals were assigned to age groups: 0 - 19 years, 20 - 29 years, 30 - 39 years, and 40 - 49 years, as some ages were poorly represented and in some individuals the age had been estimated. BMI was calculated and individuals assigned as underweight <18.5, normal 18.5 - 25, and overweight or obese >25, as specified by the World Health Organization.[23] For children less than 2 years old, baby percentile charts were used to determine BMI.

Results

A total of 54 individuals displayed evidence of macroscopic pathology from fatty streaking (41) to more advanced atherosclerotic lesions (11); 35 were black, 17 coloured, and 2 white. Ages of individuals with fatty streaks ranged from 14 to 47 years, and with atheroma from 22 to 46 years. There was a significant difference in prevalence of macroscopic lesions ($p<0.05$) between coloured (54%) and black (31%) individuals.

The age category with the lowest percentage of macroscopic pathology (20%) was the 0 - 19-year group, and the highest (44%) was the 20 - 29-year group. Black individuals in the 0 - 19-year group showed the lowest percentage (16%) of pathology, while the highest percentage (39%) was in the 20 - 29-year group. Macroscopic pathology was observed in 40% of coloured individuals in the 0 - 19-year, 57% in the 20 - 29-year, 45% in the 30 - 39-year and 62% in the 40 - 49-year group.

The highest levels of macroscopic pathology (46%) were found in overweight individuals, with a significant difference ($p<0.05$) between normal BMI and overweight males.

Regarding race and BMI, 27.3% of normal and 38.2% of overweight black individuals showed evidence of macroscopic pathology, and 47% of normal BMI coloured individuals showed macroscopic pathology – almost twice that of the corresponding black group. Two-thirds of overweight coloured individuals had evidence of macroscopic pathology, as did 50% of those underweight.

A total of 95 individuals (64%) displayed macroscopically normal thoracic aorta; 77 were black, 15 were coloured, 2 were white and 1 was Asian. The age of the 95 individuals ranged from 1 month to 47 years (mean 26.5), and included the children aged between 1 month and 8 years.

Histological results were obtained for 147 of the 149 individuals (2 samples excluded owing to tissue processing problems), all of whom, including macroscopically normal samples, showed evidence of either structural changes to the vessel wall or cellular inclusions (Fig. 1). Specifically, 93% showed some form of structural change, whereas 67% showed cellular anomalies (Fig. 1). No statistically significant differences in prevalence of microscopic anomalies were found between the various categories of individuals sampled.

Thickening of the tunica intima, disruption of the smooth muscle cells in the tunica media and branching and 'splitting' of elastic fibres with deposits of collagen in the region between the two layers can be seen in Fig. 2. Fragmentation of elastin fibres was present, to varying degrees, in individuals of all ages including children.

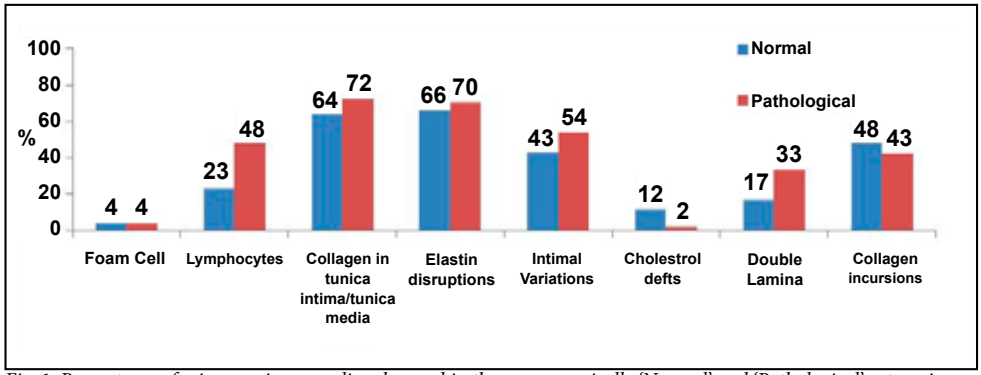

Fig. 1. Percentages of microscopic anomalies observed in the macroscopically 'Normal' and 'Pathological' categories.

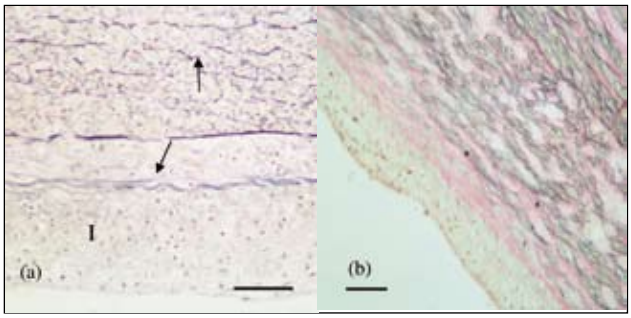

Fig. 2. (a): Fragmentation and 'splitting' of elastin fibres (arrows), with thickening of the tunica intima (I) in a 26-year-old black male (x10 EVG). (b): Increased collagen (pink) in the tunica intima in a 20-year-old black male (x10 EVG). Scale bar=100 μm.

In some samples, the collagen deposits were extensive, extending from the tunica adventitia into the tunica media, and were typically seen in association with small blood vessels (Fig. 3).

In terms of abnormal cells, foamy macrophages and lymphocytes were the most commonly observed, and were seen in macroscopically normal and pathological samples. Lipid pools and cholesterol clefts, formed by deposits of lipid in the vessel wall, were also observed in the tunica intima of both categories.

Acid mucopolysaccharide deposits were found in macroscopically normal and pathological samples. Sites of deposition appear to correspond to areas in which elastin fibres and smooth muscle cells in the tunica intima and tunica media were disrupted (Fig. 4), and were found in all age groups, including infants less than a year old.

Discussion

Our findings indicate that prevalence of macroscopic pathological lesions in the thoracic aorta is significantly higher in the coloured population than in the other population groups examined. These results, consistent irrespective of age and BMI, support the findings of the 2006 study[12] and suggest that coloureds may be at greater lifetime risk of developing atherosclerotic disease.

The higher prevalence of atherosclerosis in the coloured population may be attributable to several factors. Familial hypercholesterolaemia

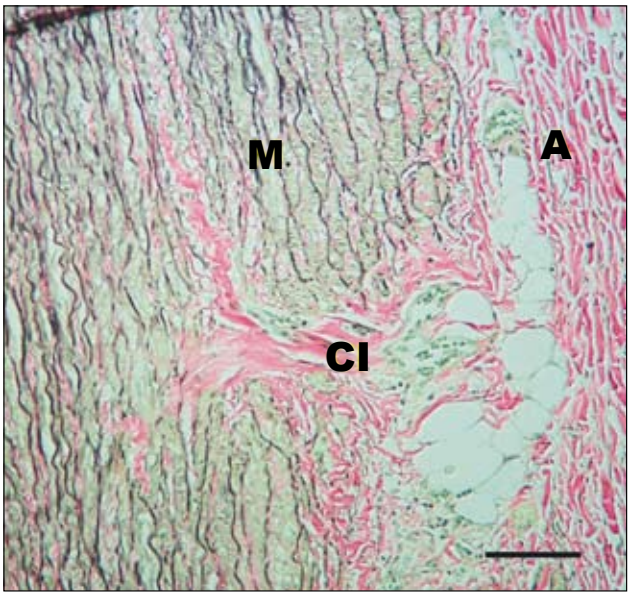

Fig. 3. A collagen 'incursion' (CI), extending from the tunica adventitia (A) into the tunica media (M), in an 18-year-old coloured male (x10 EVG). Scale bar=100 μm.

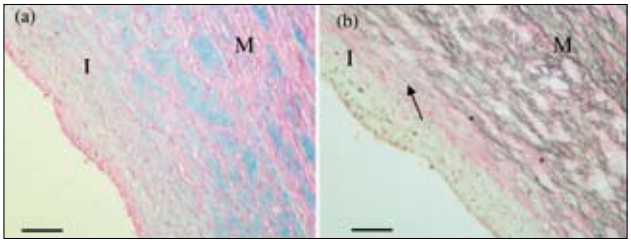

Fig. 4. (a) Mucopolysaccharide deposits (blue) in the tunica intima (I) and tunica media (M) of a 20-year-old black male (x10, PAS-AB). (b) Elastin fibre disruption and increased collagen deposition (pink, arrow) at the same site (x10 EVG). Scale bar=100 μm.

that predisposes individuals at a young age to the development or accelerated progression of CVD is a relatively common disorder in South African coloured and white Afrikaner populations[9] and could account for the presence of advanced atherosclerotic lesions in coloured individuals below 50 years of age.[12] Coloured men and women have the highest levels of cigarette smoking in South Africa – a known risk factor for CVD.[13] In the Western Cape, there is high and increasing consumption of methamphetamine hydrochloride (crystal meth), known locally as Tik. Most Tik users are coloured males under the age of 20 years.[14] Tik use among coloured females is reportedly higher than among their black counterparts.[15] Tik has been linked to high blood pressure, irregular heartbeat and damage to blood vessels of the brain, resulting in stroke,[14] and is implicated in other cardiovascular conditions such as aortic dissection, coronary artery atherosclerosis, and aortic degenerative changes.[16] Abuse of Tik can lead to high-risk sexual behaviour, exposing users to greater risk of HIV infection.[14,16] The age group most at risk for HIV infection in sub-Saharan Africa is that of 20 - 39 years.[17] HIV and antiretroviral treatments (ART) have both been associated with pathological changes in the cardiovascular system, including atherosclerosis.[7,16,18] Our high prevalence finding of atherosclerotic lesions in coloured and black individuals in the 20 - 29-year age group suggests that some might have been HIV-positive or receiving ART.

A positive association was found between BMI and vascular pathology in the black and coloured groups. Many individuals with atherosclerotic plaques were overweight, although a high prevalence of lesions was also found in coloured individuals with a normal BMI. Obesity is a major risk factor for CVD,[4] so this association, together with the fact that obesity is increasing in South Africa,[19] is perhaps not unexpected. Increasing obesity levels in the black population have been linked to urban migration and the adoption of a diet that is higher in dietary fat.[19,20]

Almost 90% of individuals in this study were positive when analysed for the presence of acid mucopolysaccharides, which have been linked to non-atherosclerotic CVD such as intimomedial mucoid or cystic medial degeneration.[21] Acid mucopolysaccharides have been associated with damage to elastin and an increase in collagen in the tunica intima and the tunica media,[22] as frequently observed in this study, and which can result in vessel wall dysfunction.

The overall levels of macroscopic pathology found in the thoracic aorta were, however, lower than expected; 64% of individuals showed no signs of pathology, in contrast with other studies[4] that reported the virtually ubiquitous presence of early atherosclerotic lesions among younger people. However, our study did not include examination of the coronary arteries or the abdominal aorta, where lesions are also common.[4]

The widespread presence of microscopic structural disruptions in the aortic wall across all age and racial categories and in macroscopically normal individuals does, however, suggest that these changes are physiological rather than pathological in nature. Disruptions to elastin and high densities of collagen, for example,

occur in response to haemodynamic forces and may be related to vessel wall repair processes.[23] Affected areas might, however, function as nidi for the subsequent development of lesions in the vessel wall.

Limitations and conclusions

Our findings are based on a small number of individuals and are not representative of the wider South African community. Further investigations are needed into the cardiovascular health of South Africans, in particular more targeted autopsy studies that include larger numbers of females and less well-represented racial groups such as Asians, and white Afrikaners in whom familial hypercholesterolaemia is well documented. Future studies could expand data collection to include drug, HIV and genetic testing, and a detailed family history. Given the health care system demands, it is unlikely that such information could be collected at the primary health care level, to which most South Africans are restricted. Health care workers can be alerted to the potential underlying conditions such as hypercholesterolaemia in the coloured population. The abuse of tik can exacerbate existing cardiovascular conditions and result in higher mortality rates among users. Young people in particular must be educated about lifestyle-associated risk factors and so avoid or delay the onset of associated heart disease, aneurysms and strokes.

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CHRIS BARNARD'S CAROL

(Trad. carol)

God rest ye merry gentlefolk
Let this not ye dismay
We know you're sorely grieving
That Chris retires today
Tis not the first and only time
That he has gone astray
So its tidings of comfort and joy

From lowest Louw to Lillehei
I ask you to express
Resounding praise your glasses raise
Die seun van Beaufort Wes
Together let's retell the tale
His honours list, each prize
Even if I glaze your weary eyes

Rude, reckless, poor, untaught came he
Tough surgeons of repute
Ejected him To City
Refuge of the destitute
A master's followed in a trice
Then an MD on TB
You'll find out all the rest in his CV

Lovingly he cut and stitched
Ah love indeed and bliss
Nurses by the dozen they all
Testified to this
Gutless little puppy dogs
Resulted in awards
Invitations and Open-hearted hordes

Then came the day that you all know
Each foe his teeth did gnash
Silver Crosses, Golden Medals
Cordon Bleu with Sash;
How they lauded him in London,
Spain and Equador,
Andropov reads his books and asks for more.

His lot was not to fear rejection,
Obliged by stars and screen,
Sophia, Gina, grace, Ms heelal
P'haps thatcher or the Queen.
If you ask what's left to conquer
Turn on the TV,
Read the Monday morning paper
and you'll see.

Professor C Barnard's Retirement Party – 1983

Maurice Kibel