

HUMAN CORONARY ARTERIES- A STUDY BASED ON MICROSCOPY

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ABSTRACT

BACKGROUND

The coronary arteries are the biggest vasa vasora in the body since the heart is considered to be a modified blood vessel. The increased myocardial oxygen demand of the heart is met wholly by two coronary arteries. Hence, patients with coronary artery disease are prone to develop myocardial ischemia. The study is aimed at the structural changes of these vessels in the population of middle Kerala of various age groups from birth to seventy years.

MATERIALS AND METHODS

The specimen for histological study were fixed in 10% formalin and subjected to tissue processing. The sections were taken at the thickness of 5 microns and stained by using Haematoxylin- Eosin method and Verhoeff's method for elastic fibers. The thickness of arterial wall were measured using ocular micrometer.

RESULTS

Structural changes of the three layered vessel walls were observed. Intimal proliferation, splitting, fragmentation and reduplication of internal elastic lamina were the important histological observation in the arterial wall as age advances. It was observed that there was a sixty fold increase in the thickness of intima from foetal to sixth decade of life. Tunica media exhibit a six fold increase in thickness probably due to muscular hypertrophy. The tunica adventitia have a threefold increase in thickness which least compared with other tunics.

CONCLUSION

These changes may favour atherosclerosis resulting in myocardial ischemia. Ischemic heart disease is the major cause of death and disability among the age group of third to sixth decade of life.

KEYWORDS

Coronary Artery, Microscopy, Tunica Intima, Tunica Media, Tunica Adventitia.

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BACKGROUND

Study on coronary arteries in relation to area of distribution and existence of an intercoronary anastomosis is obviously a matter of prime medical importance. This study is an attempt to throw light upon the microscopic structural changes of right and left coronary arteries in different age groups. The basic structure of coronary arterial wall consists of tunica intima, tunica media and tunica adventitia from within to outward. The tunica intima is the innermost layer formed by endothelial cells resting on a basement membrane and become evident at birth.¹ Internal elastic lamina separate the intima from media. Media consists of alternating layers of smooth muscle cells, collagen and elastic fibers. External elastic lamina separates the media from tunica adventitia. Adventitial layer is composed of

dense fibrous tissue in which embedded the blood vessels, connective tissue cells, nerves and lymphatics. Intimal proliferation is the important change noticed in the arterial wall as age advances.² The amount and structural organisation of elastic and muscular tissues of the thickened intima are strongly influenced by size, length and mode of ramification of parent of coronary tree.³ The changes that developed with the aging may lead to atherosclerosis.⁴

MATERIALS AND METHODS

Human heart specimens for this study was collected from postmortem cases brought to forensic Medicine department of Govt. Medical College, Kottayam and foetal hearts from Labour room of Obstetrics & Gynaecology department after getting ethical clearance. The tissues for the histological study are taken from both left and right coronary close to its origin.

The specimens for histological purposes were transferred to 10 percent formalin, the fixative solution, immediately after the removal.

Forty two heart specimens were made use for this study. The specimens were grouped according to the age (Table II). The fixed specimens are subjected to the tissue processing. The sections were taken at a thickness of 5

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microns. Sections stained with H & E and Verhoeff's staining technique and observed under low power microscope. The thickness of the arterial wall measured by using eye piece micrometer calibrated against stage micrometer.

Staining Methods Used

1. Haematoxylin – Eosin method.
2. Verhoeff's method for elastic fibers.

Verhoeff's Method

Staining Solution-

- | | |
|------------------------------------|-------|
| 1. Stock 5% alcoholic haematoxylin | 20 ml |
| 2. 10 %Ferric Chloride | 8 ml |
| 3. Verhoeff's iodine | 8 ml |

Verhoeff's Iodine-

- | | |
|--------------------------------------|--------|
| 1. Saturated solution of picric acid | 100 ml |
| 2. Acid Fuchsin | 10 ml |

Staining Method-

1. Bring sections to water.
2. Stain for 15-30 minutes until get black.
3. Differentiate in 2% Ferric Chloride until elastic fibers are clearly seen. Rinse in water and examine under low power microscope.
4. Wash in water, then 95% alcohol to remove iodine staining.
5. Wash in water for 5 minutes.
6. Counter stain with Vanhoeff's stain for 3 minutes.
7. Dehydrate, clear and mount.

Elastic fibers and nuclei appear black to blue black in colour. Cytoplasm of muscle is appear yellow colour and collagen fibers appear red. The sections are taken from both coronary arteries and studied the structural alterations in different age groups.

Sl. No.	Period	Male	Female	Total
1	Foetal	7	1	8
2	First decade	3	1	4
3	Second decade	2	2	4
4	Third decade	3	2	5
5	Fourth decade	4	4	8
6	Fifth decade	5	2	7
7	Sixth decade	2	1	3
8	Seventh decade	2	1	3
	Total	28	14	42

Table I. Number of Specimens Collected from Various Decades of Life for Microscopic Study

OBSERVATIONS

Histological observations are based on Haematoxylin and Eosin stained sections and Verhoeff's elastic staining technique. The thickness of the triple layered arterial wall were measured using ocular micrometer under low power. It was observed that at birth the tunica intima is very thin and formed by a single layer of endothelial cells resting on a basement membrane. The internal elastic lamina is very prominent, which is a continuous thick layer showing tortuosity (Figure 1). The thickness is equal in both coronary arteries. Tunica media and adventitia are more thicker than tunica intima (Table II). In the first decade of life tunica intima remains thin but the thickness of tunica media and adventitia were increased. Tunica media contains smooth muscles, elastic fibres and collagen fibres. Tunica adventitia were made up of loose connective tissues and blood vessels. Internal and external elastic lamina were intact. (Figure 2). In the second decade the intimal proliferation begins with subendothelial connective tissue deposition. The tunica media and adventitia remains without much changes (Figure 3). The third decade onwards the intimal proliferation becomes marked and more in the left coronary artery. Subendothelial layer consists of collagen fibre, smooth muscle cells and elastic fibres. The smooth muscle cells migrate from tunica media to tunica intima and intima becomes stiffest and prominent layer. In the fourth decade onwards intimal proliferation well marked and more on the left coronary. Intimal cushions were formed and projecting into the lumen which contains smooth muscles, elastic fibres, collagen fibers and other connective tissue cells (Figure 4). Internal and external elastic lamina begin to get disrupted (Figure 5). Tunica media almost get merged with the intima. Tunica adventitia becomes thin. In the fifth decade intimal proliferation is going on, internal elastic lamina is duplicated and intima appears to be double layered (Figure 7). In the sixth and seventh decades increase in the thickness of the intima of both coronary arteries and focal splitting of elastic lamina can be appreciated (Figure. 8). The thickness of media is more compared with that of adventitia. It is observed that the tunica intima and media having no blood vessels their own. As the age advances there is 60 fold increase in the thickness of intima was noticed with twelve fold increase in the total thickness (Chart I and II). The gross effect of these changes lead to the stiffness of arterial wall, reduction of expansile property, and luminal diameter of the vessel. Intimal smooth muscle infiltration and focal splitting of elastic lamina can lead to atherosclerosis which in turn result in ischemic heart disease.

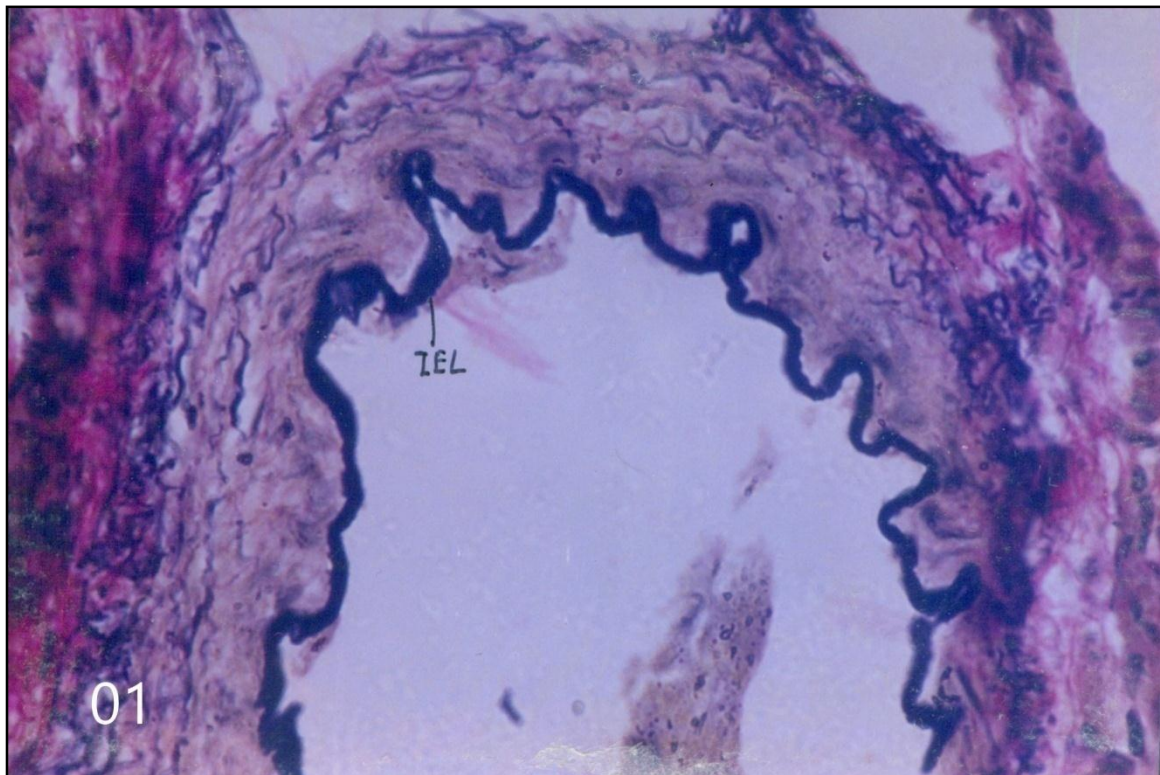


Figure 1. T.S. of Left Coronary Artery of a Full Term Foetus. Internal as well as External Elastic Lamina were seen. Elastic fibres Scattered in the Tunica Media. Verhoeff's 400 x

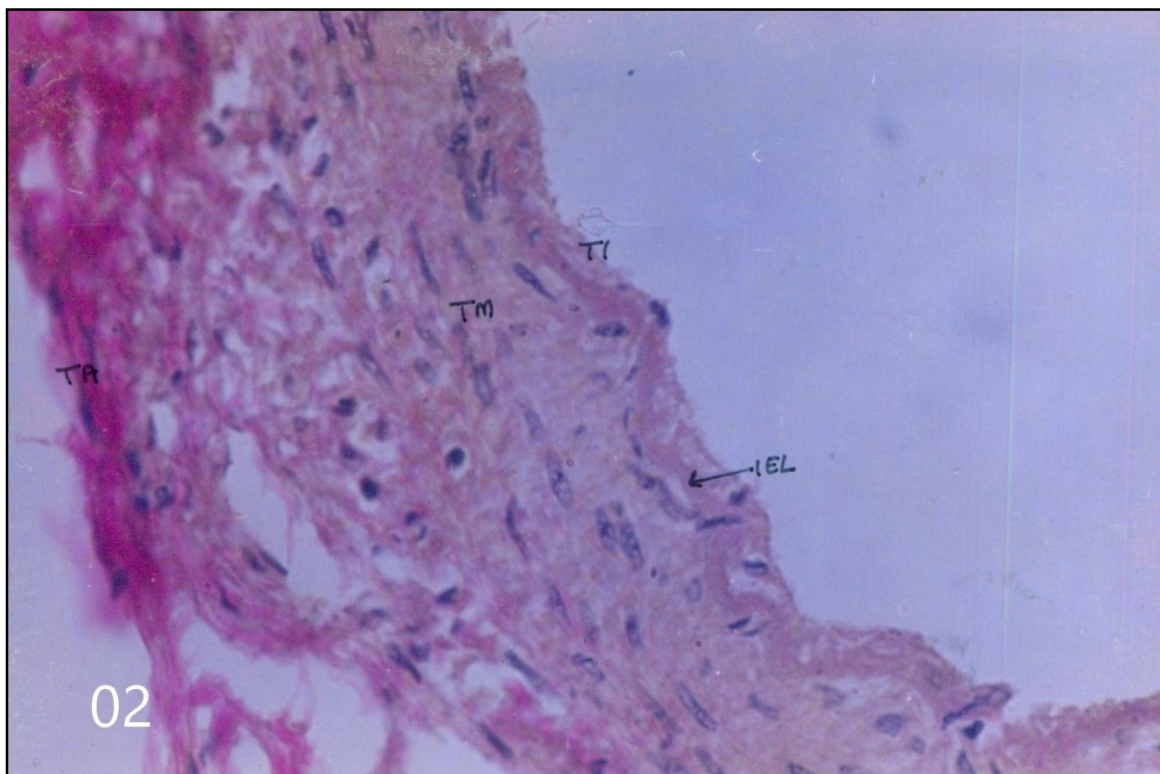
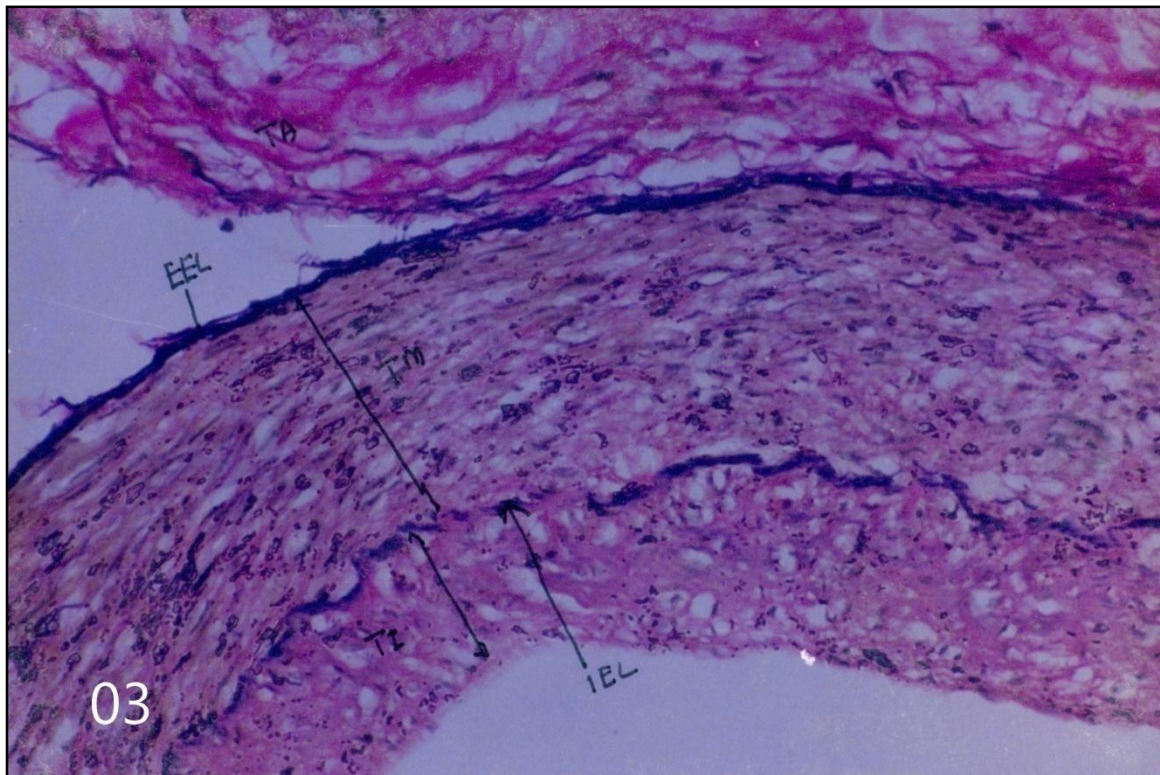
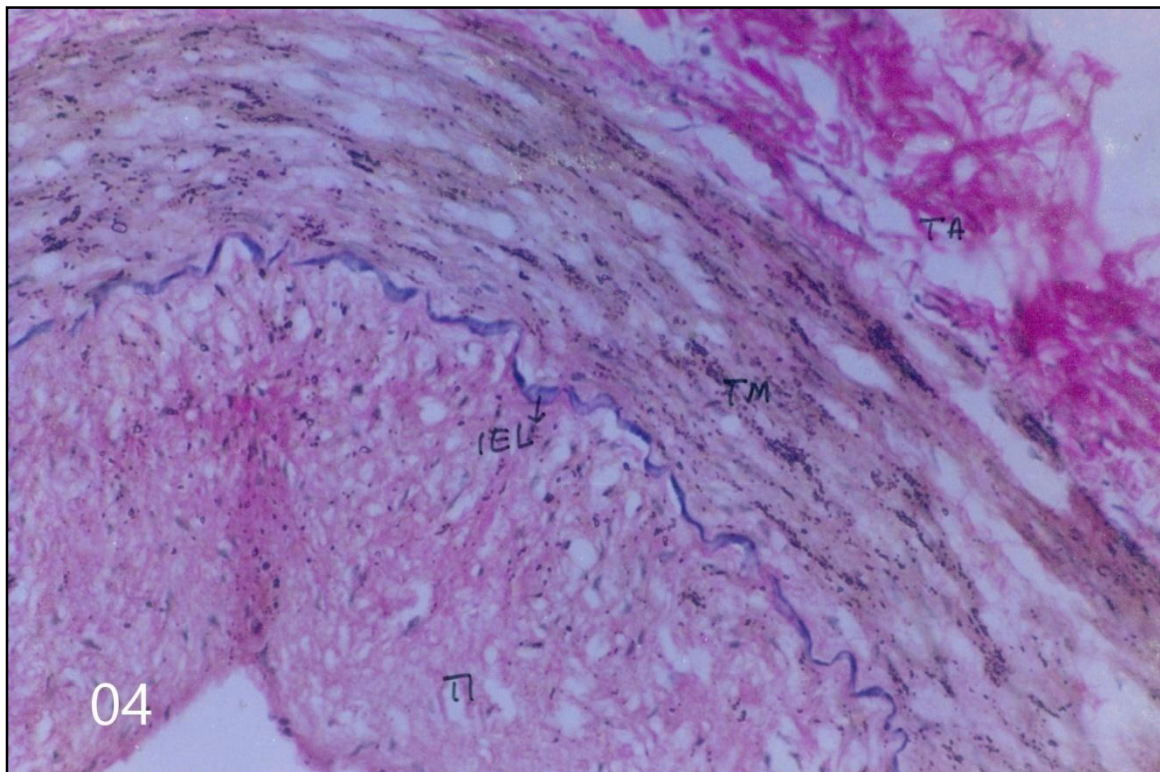


Figure 2. T. S. of Right Coronary Artery of a 7 year old Child. Verhoeff's. 400 x



**Figure 3. T. S. of Left Coronary Artery of a 20 year old Male.
Tunica Intima is thick. Verhoeff's. 200 x**



**Figure 4. T. S. of Left Coronary Artery of a 35 Year old Male Showing Smooth
Muscle Infiltration into Tunica Intima. Verhoeff's. 200x**

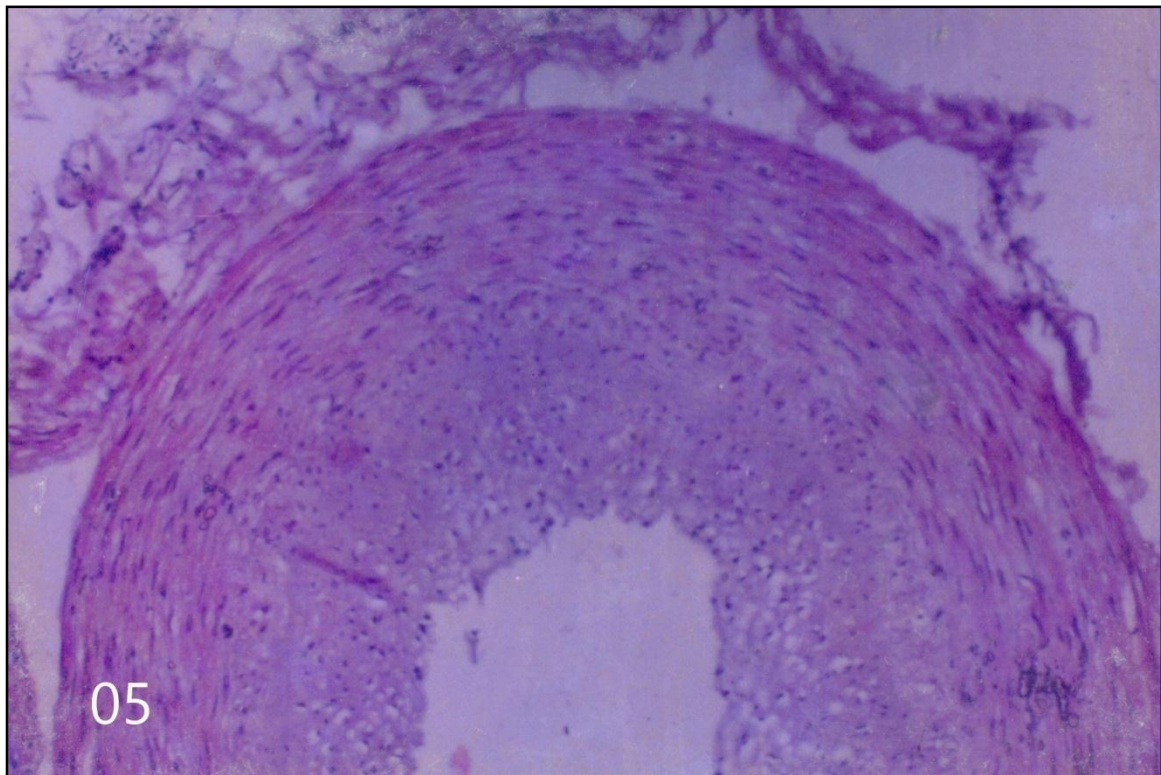
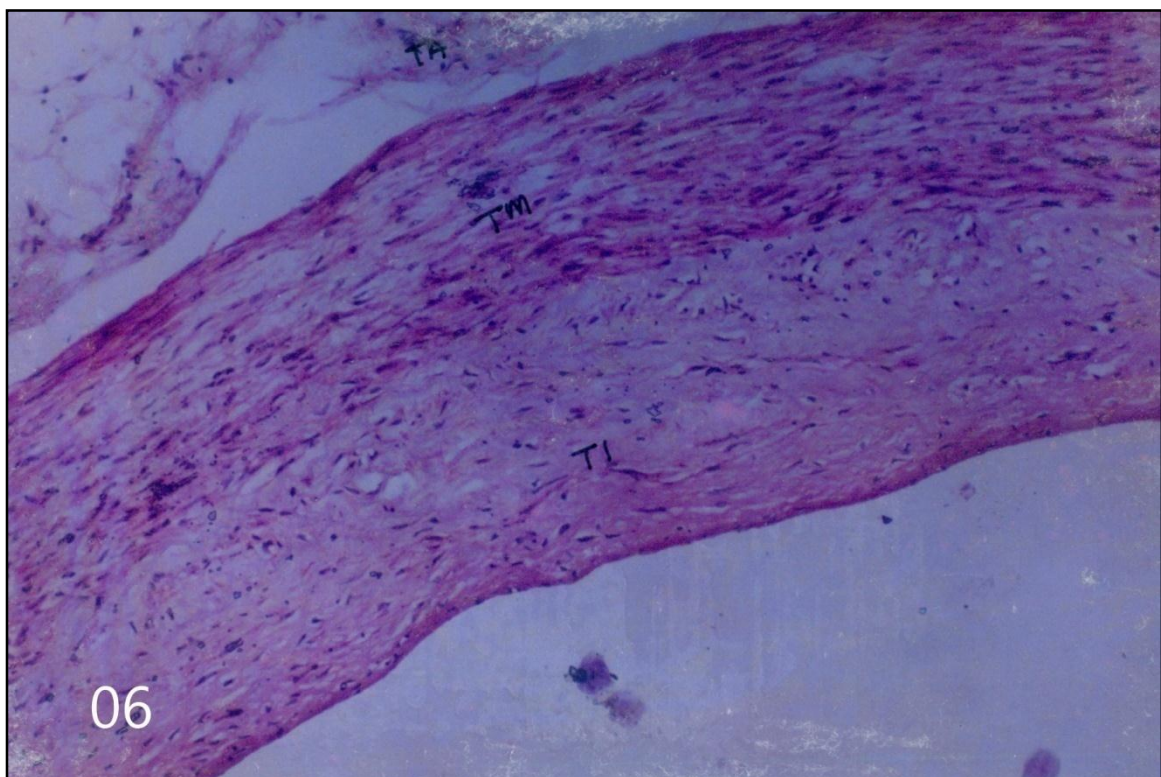


Figure 5. T. S. of Right Coronary Artery of a 40 Year old Male. H/E. 100 x



**Figure 6. T. S. of Right Coronary Artery of a 50 Year old Male.
Tunica Intima is the Prominent Layer. H/E. 100x**

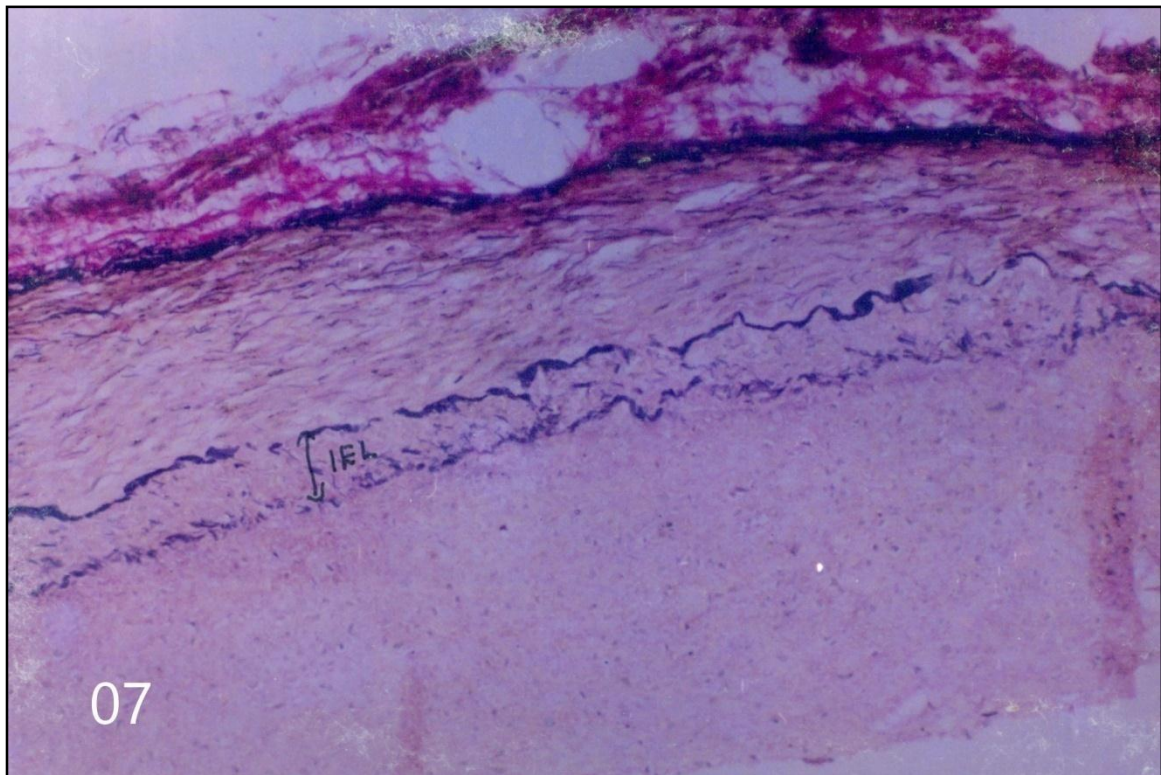


Figure 7. T. S of Left Coronary Artery of a 60 Year old Male. Internal Elastic Lamina Duplicated. Intima consist of Two Layers. Verhoeff's. 100x

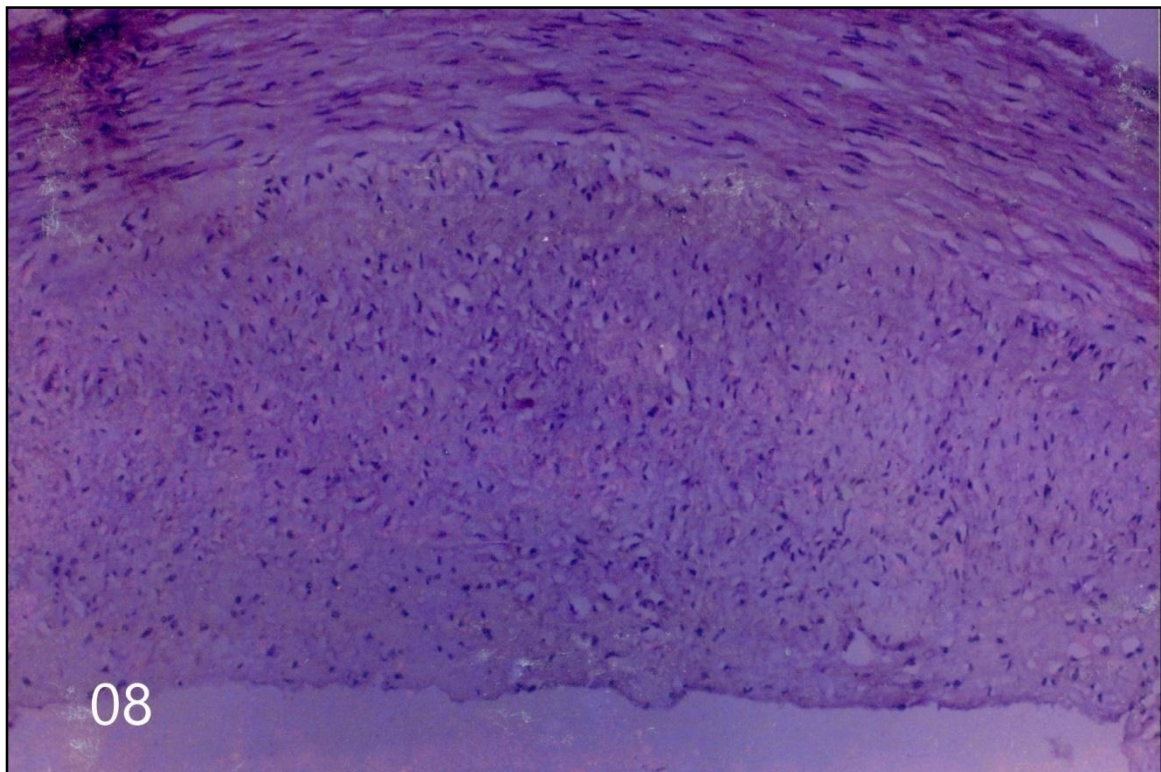


Figure 8. T. S. of Left Coronary Artery of a 70 Years old Male. Tunica Intima is very Thick. H/E. 100 x

Age (Y)	Sex		Thickness of Intima (µm)		Thickness of Media (µm)		Thickness of Adventitia (µm)		Total Thickness (µm)	
	M	F	Right	Left	Right	Left	Right	Left	Right	Left
Foetal	7	1	10	10	60	82	80	70	150	162
0-10	3	1	15	10	200	200	250	250	465	460
11-20	2	2	135	110	200	200	200	200	535	510
21-30	3	2	200	300	175	175	175	200	550	675
31-40	4	4	225	300	375	325	200	200	800	725
41-50	5	2	375	450	375	366	250	250	1000	1066
51-60	2	1	600	650	400	400	200	200	1200	1250
61-70	2	1	600	675	400	400	200	200	1200	1275

Table II. Showing Thickness of Coronary Arterial Wall in Various Age Group

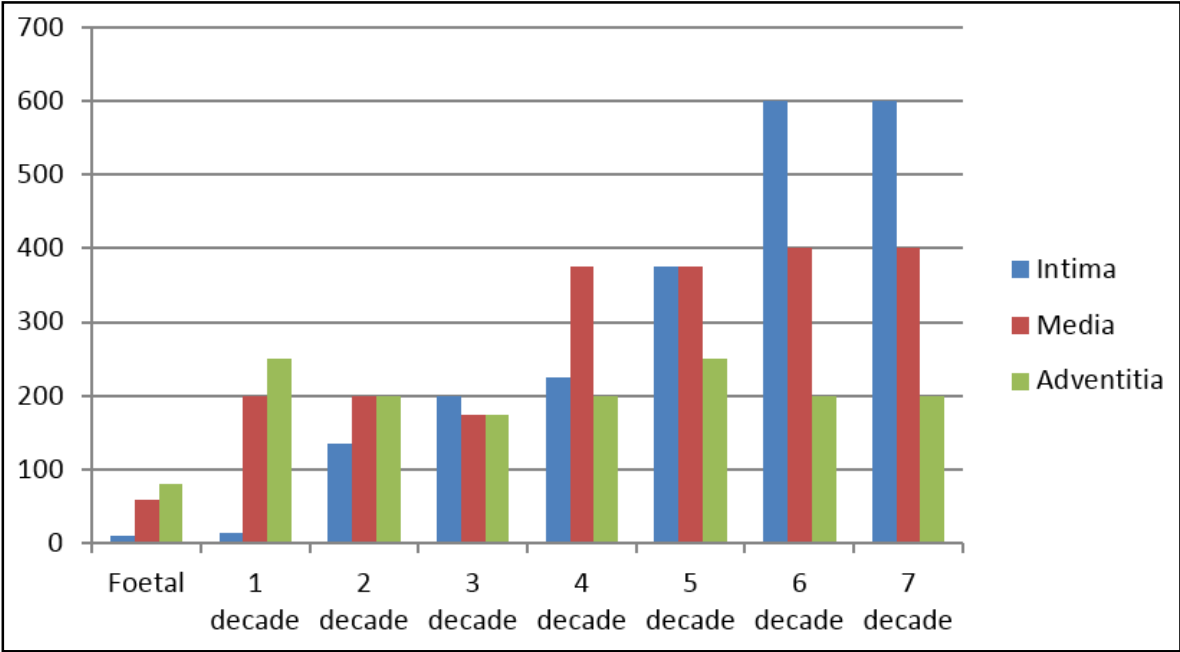


Chart 1. Showing Changes in Thickness (in Micron) in the Right Coronary Arterial Wall

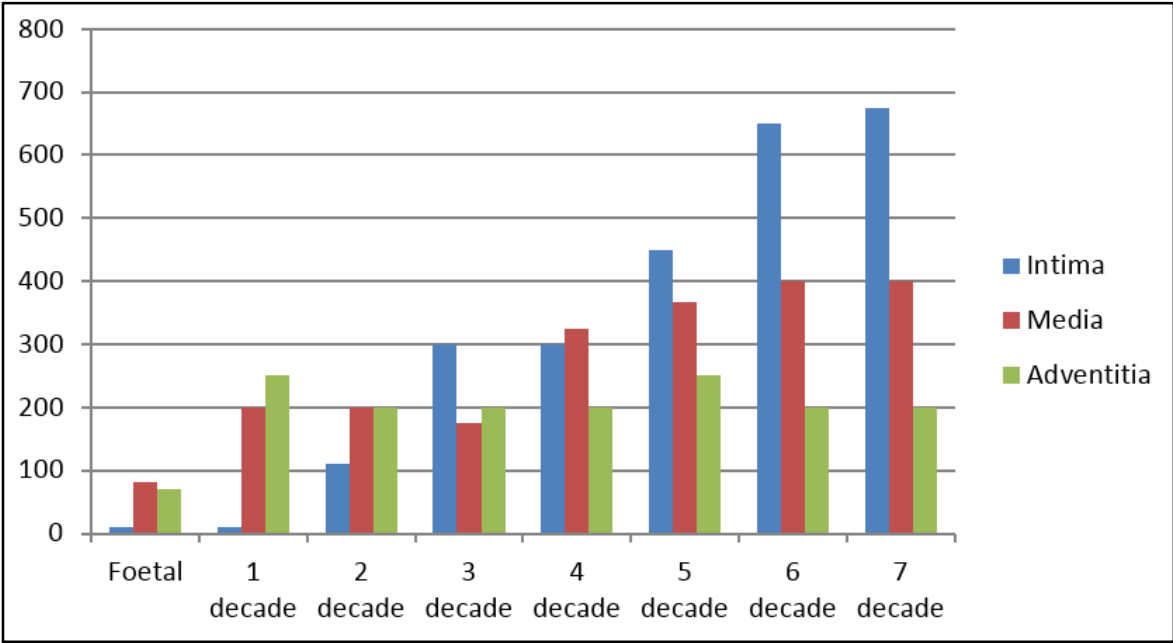


Chart II. Showing Changes in Thickness (in Micron) in the Left Coronary Arterial Wall

DISCUSSION

Microscopic study of coronary arterial wall has been done extensively in different age groups by many workers. Earlier it was assumed that the sex differences were significant factor to explain the variability of coronary wall thickness. The male infants having a thicker intima than female one.⁵ The micro architecture of human coronary artery compared with other muscular arteries, the characteristic feature is the rapid development of intimal proliferation which is a progressive phenomenon. In the present study this is evident from adolescence age group onwards. There is a slow postnatal increase in the number of smooth muscle cells of tunica media of the coronary artery and it is compensated by intimal proliferation.

In this study, it was observed that intimal proliferation begins to occur in the first and second decade of life, and it became more pronounced in the third decade especially in the left coronary artery. In later decades this layer proliferates and become the thickest of all 3 layers (Chart I and II). This micro structural change reflects the intimal response in connection with ageing process. Holzapfel et. al. (1998) remarked that intima has significant load bearing capacity and mechanical strength compared with other tunics.⁶ The intimal thickening was associated with reduction of luminal diameter resulting in reduced blood flow and myocardial ischemia.

The intimal thickening sometimes forms pads or cushions, projecting in to the lumen. This cushions contains smooth muscles, elastic fibres, collagen fibres and connective tissue cells. These changes in the intima may favour the development of atherosclerosis. It was reported that (Velican and Velican, 1981, 1985) the intimal thickening of coronary artery is gender and branch anatomy dependant. The vessel size and arterial bed also affect the intimal thickening. In this study it was found that the intimal proliferation is more in the left coronary artery. This may due to its wider caliber, unique branching pattern and wide area of distribution compared to right coronary.

In this study it was seen that the tunica media shows progressive thickening in both coronaries starting from the first decade of life. As age advances there occurs fibrosis, reduction in number of smooth muscle, splitting of internal elastic lamina and tunica media seems to merge with the tunica intima. Tunica adventitia shows slow and steady

increase in thickness but it was less compared with the other two tunics (Table 2). All these findings in the arterial wall have attributed due to increased strain or wear and tear of ageing or physiological remodeling of arterial wall.⁷

CONCLUSION

Coronary atherosclerosis is the major cause of death and disability in developed countries. CAD is caused by plaque buildup in the coronary artery wall eventually leads to narrowing of lumen of vessel. Structural changes undergone by coronary arterial wall was studied in 42 hearts of varying age groups in both sexes. It was found that intimal proliferation become evident from 2nd decade onwards. Left coronary artery shows more intimal thickening from 3rd decade onwards and it progressively increases as age advances. Intimal proliferation, smooth muscle infiltration and medial sclerosis of tunica media are important findings which predispose to atherosclerosis and eventually myocardial ischemia.

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