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Original Research Article

Comparison of microanatomy of ascending aorta and pulmonary trunk with age: A cross-sectional study

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ABSTRACT

Background: Cardiovascular disease (CVD) accounts for the leading cause of mortality worldwide and it has a strong association with age. Aging brings about structural and functional changes in vessels, culminating in CVD. This study aims at determining and comparing the structural age changes in the two great vessels of heart, ascending aorta (AA) and pulmonary trunk (PT).

Materials and Methods: Human ascending aorta and pulmonary trunk samples were obtained during autopsy from 55 individuals of different age group. After processing, they were stained with eosin haematoxylin and special stains to identify connective tissue fibres and smooth muscle cells. Thickness of each tunic of the vessel wall, full wall thickness, quantity of smooth muscle cells and severity of fragmentation of elastic fibres were detected.

Results: In all age groups, PT was thinner than AA. Average full wall thickness of AA was greatest in the sixth decade of life while that of PT in the fourth decade. Elastic fibres were long, straight and arranged in a lamellar pattern in tunica media of both the vessels in fetal life. They underwent fragmentation from first decade of life in PT and from third decade in AA. Grade5 fragmentation was noticed only in PT. Degeneration of smooth muscle cells occurred in both the vessels with age, but was very little in PT.

Conclusion: Both the great vessels showed degenerative changes with advancing age, but after the first decade of life, the changes were very less in PT when compared to AA.

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1. Introduction

Cardiovascular diseases (CVDs) are the major contributors to mortality and morbidity worldwide.[1] Aging is an unavoidable risk factor leading to CVDs.[2] Arterial stiffness is considered as the index of vascular aging and the elastic arteries proximal to the heart are more sensitive to the effects of age.[3] The functional stiffness of an artery depends upon the intrinsic properties of the materials constituting its wall and the relative thickness of the vessel wall with respect to its lumen.[4] The collagen fibres, smooth muscle cells, and elastic fibres make up the primary load-bearing framework of the vessel.[5] The new treatment modalities for CVDs aim at reducing or reversing the structural changes that bring about vessel stiffness.[6] The present study aims at describing and comparing the changes in vessel wall thickness, elastic fragmentation, amount of collagen fibres and smooth muscle cells in the walls of ascending aorta (AA) and pulmonary trunk (PT) across different ages.

2. Materials and Methods

The ascending aorta and pulmonary trunks were obtained during autopsies conducted at Government Medical College, Thiruvananthapuram. Sample collection was done over a period of 1 year after getting institutional ethics

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2.1. Sample size

Fifty five samples were collected; two of them belonged to the fetal group (one of 32 weeks and another of 34 weeks). To avoid autolytic changes, all samples were collected within six hours of death. Deaths due to crush injuries of chest, burns, or as a result of cardio pulmonary causes were excluded from our study. Individuals of both sexes up to eighty years were categorized into 9 groups.

2.2. Methodology

Rectangular tissue bits were collected from the anterior walls of AA and PT, almost two centimetres distal to aortic and pulmonary valves respectively. Thereafter, specimens were immediately transferred to 10% formalin for fixation. 24 hours after fixation, routine histological processing[7] was carried out in the histology lab of Anatomy Department, Government Medical College, Thiruvananthapuram. Serial sections of 6 microns thickness were taken using a rotary microtome. Hematoxylin and eosin staining was done after incubating the sections for one hour. Orcein Van Gieson's and Masson's trichrome special stains were used to visualize the elastic fibres, collagen fibres, and smooth muscle cells in the vessel wall. The sections were mounted and examined under a binocular microscope. The thickness of the entire vessel wall as well as that of each tunic, the severity of fragmentation of elastic fibres, number of smooth muscle cells, and amount of collagen was noted.

Using an eyepiece micrometer, under x10 objective, the thickness of each tunic of the vessel wall was measured. Measurements were made in three different fields and the average value was determined. The portion of the vessel wall from internal elastic lamina to the outer limit of muscle layer was considered as the thickness of tunica media. Fragmentation of elastic fibres and number of smooth muscle cells/ per 100 mm^2 in tunica media were determined at a magnification of x40. Focal breakup of elastic fibre in tunica media of the vessel wall was regarded as elastic fragmentation.[8] Grades were assigned for the severity of elastic fragmentation as per Schlatman and Becker[8] criteria. A microscopic field with maximum fragmentation was considered for grading. $\leq$ 2 foci of elastic fibre breakup in one field were considered as grade 1, 3-10 foci of breakup as grade 2, $\geq$ 10 foci of breakup as grade 3, if one third to one half of the vessel wall showed fragmentation with loss of lamellar pattern, it was considered as grade 4 and if the fibres underwent severe fragmentation throughout the vessel wall with complete loss of lamellar pattern, it was assigned grade 5.

Smooth muscle cells were counted using a net micrometer with a 10x10mm square grid. Five random areas were selected and the average number per 100$mm^2$was calculated under a high-power lens (x40 objective and x10 eyepiece).

2.3. Statistical analysis

Data were entered in Microsoft Excel and analyzed using the SPSS version 20.0. Quantitative variables were described by mean, standard deviation, minimum and maximum. Qualitative variables were described by percentage distribution. Comparison of quantitative variables involving more than two groups was done by Analysis of Variance (ANOVA). For comparison between two groups, quantitative variables were analyzed by independent sample 't-test. A 'p-value of 0.05 was taken as the level of significance.

3. Results

The specimens obtained for microscopic study were divided into 9 groups according to age, as depicted in Table 1.

Table 1: Age distribution

Group No.	Age Groups	No. of specimen
1	Fetus	2
2	0 – 10	2
3	10 – 20	4
4	21 – 30	8
5	31 – 40	6
6	41 – 50	7
7	51 – 60	9
8	61 – 70	11
9	71 – 80	6

3.1. Tunica intima

In group 1(foetal group) of both AA and PT, subendothelial connective tissue was absent. In group 2 onwards, it appeared between internal elastic lamina (IEL) and endothelium. There was a consistent increase in thickness of tunica intima of both the vessels with advancing age and it was statistically significant ($p<0.001$). For AA, the mean intimal thickness was $6.4\pm1.8\mu$ in group 1, and it rose to $267.9\pm32.9\mu$ in group 9. For PT, the mean thickness increased from $1.8\pm0.6\ \mu$ in group1 to $149\pm11.6\mu$ in group 9.

3.2. Tunica media

Compared to AA, tunica media of PT was thinner in all age groups. The ratio of the thickness of tunica media of PT to that of AA (P/A ratio) was found to be between 0.5 and 0.8 in all age groups except in group 1, where it was nearly 1.

3.3. Tunica adventitia

In Group 1 of AA, the mean thickness of tunica adventitia was 281.5±37.5μ (42.5% of full wall thickness), while in PT, it was 172.6±13.3μ (32% of its full wall thickness). After 30 years, a reduction in the proportion of tunica adventitia was observed in both vessels. It declined to 18.45% and 20.9% in group 9 of AA and PT respectively.

3.4. Full wall thickness

The average full wall thickness of AA was greatest in the sixth decade. Its mean thickness in this group was 1914.1±176.8 μ (Figure 2). Student's T-test showed that in individuals <50 years the mean full wall thickness was 1618.6 ± 351.1 μ, while in those >50 years, the mean thickness was 1857.2±149 μ. The increase in thickness was statistically significant (p=0.002).

In the case of PT, the average full wall thickness was greatest in the fourth decade. It was 1204.3±32.9μ (Figure 2). Though there were statistically significant changes in full wall thickness across different age groups (p<0.001), the comparison between individuals below 50 years and above 50 years did not show any statistical significance (p=0.672).

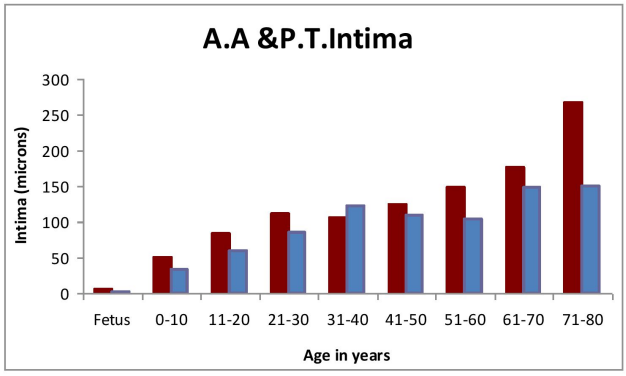

Fig. 1: Comparison of mean intimal thickness of ascending aorta (red) and pulmonary trunk (blue) in different age group

3.5. Elastic fibres

In AA, internal elastic lamina (IEL) was continuous in groups 1 and 2 (Figure 4). In groups 3 to 5, there was occasional splitting and in groups 6 to 9 there was a replication of IEL. PT showed fragmentation of IEL from group 2 onwards. Long, parallel, compactly arranged elastic laminae were seen in tunica media of groups 1 to 3 of AA. In PT, Group 1 showed compactly arranged laminar elastic fibres, while Grade 3 fragmentation was noticed from the first decade onwards. It was seen only by the sixth decade in AA. Grade 5 fragmentation was not at all seen in AA, but 17 out of 55 tissues of PT showed it. (Figure 5)

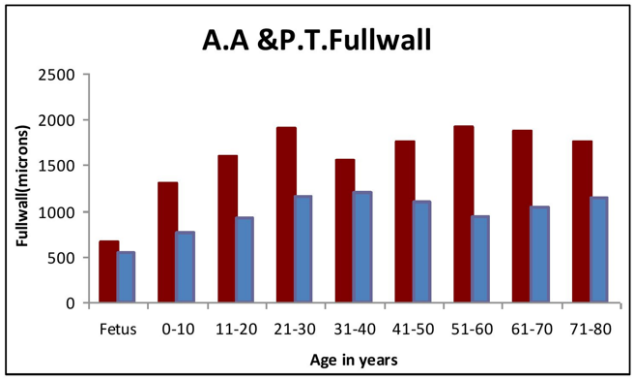

Fig. 2: Comparison of average full wall thickness of ascending aorta (red) and pulmonary trunk (blue) in different age groups

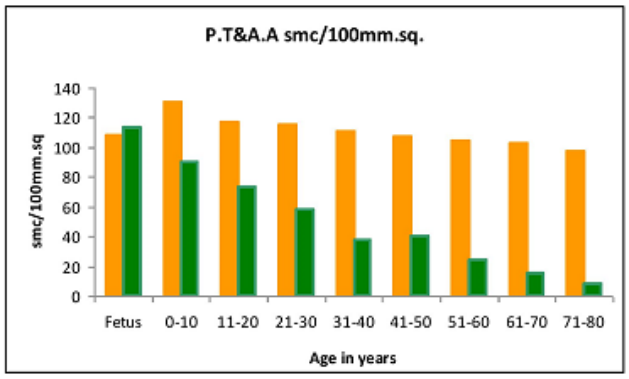

Fig. 3: Comparison of mean number of smooth muscle cells in as ascending aorta (green) and pulmonary trunk (orange)

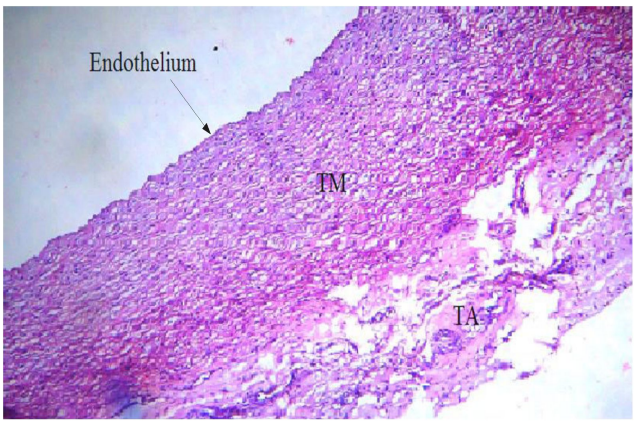

Fig. 4: Ascending aorta of a fetus of 34 weeks, showing compactly arranged, parallel to each other elastic fibres, in tunica media (TM). Nuclei of smooth muscle cells seen in between elastic lamellae. No sub-endothelial layer seen in tunica intima (TI). (H&E Staining ×100)

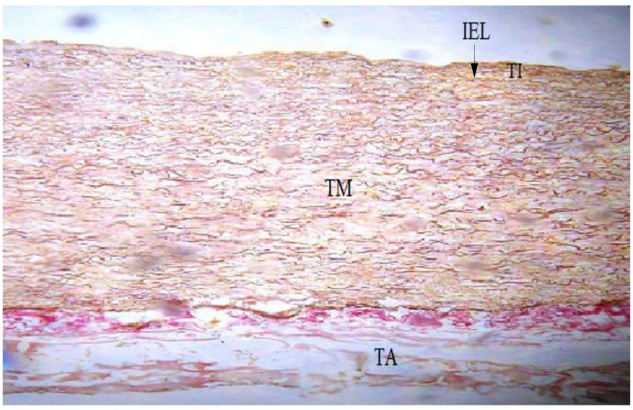

Fig. 5: Pulmonary trunk of 25 year old male showing Grade 4 frangmentation of elastic fibres (Orcein-Van gieson ×100). TI: Tunica intima, TM: Tunica media, TA: Tunica adventitia, IEL: Internal elastic lamina

3.6. Smooth muscle cells

The comparison between the number of smooth muscle cells in tunica media of AA and PT is depicted in Figure 3. In ascending aorta, the average number of smooth muscle cells was maximum in the fetal group whereas, in pulmonary trunk, it was more in the first decade of life when compared to the fetal group. Thereafter degeneration of smooth muscle cells occurred, but to a very less extent when compared to AA. In all age groups except the fetal group, PT was found to be more muscular than AA.

3.7. Collagen fibres

Quantitative collagen estimation couldn't be made, but in tissues with higher grades of elastic fragmentation and smooth muscle degeneration, it occupied all the spaces between the fragmented fibres.

4. Discussion

Though many researchers have studied the microscopic structure of AA and PT, reports of a comparative study involving both the vessels with relation to age are limited in the literature. Large artery stiffness being a determinant of cardiovascular morbidity and mortality and also taking into account their common embryological origin, a detailed study of age changes in both the vessels was carried out.

In our study, it was observed that there was a progressive increase in average intimal thickness with advancing age. Sub endothelial connective tissue was absent in foetal AA and PT, but it appeared between endothelium and internal elastic lamina by the first decade of life in both the vessels. Studies by Lakatta[9] and Crawford[10] also reported the absence of subendothelial connective tissue in ascending aorta at birth. According to Crawford, a loose meshwork of connective tissue separated internal elastic lamina and endothelium in early childhood and it increased in amount and density with the passage of years. Ross et al[11] showed that definite intimal thickening occurred in older children and invariably some stretching, splitting, or fraying of underlying internal elastic lamina occurred over years. In our study also, in AA occasional splitting of internal elastic lamina was noticed from 2nd decade onwards and replication from 5th decade while in PT splitting of internal elastic lamina started from 1st decade itself.

On comparing the thickness of tunica media of AA to that of PT, it was observed that the ratio (P/A) was within a range of 0.4 to 0.8 in all age groups except in fetal group where the P/A ratio ranged between 0.96 and 0.99. Heath et al[12] compared the thickness of tunica media of AA and PT in normal individuals and individuals with pulmonary hypertension. Individuals with pulmonary hypertension had a thick media as that of aorta and the P/A ratio was nearly 1 as in the fetal group. On comparing the thickness of tunica media of individuals above 50 years to those below 50 years, a statistically significant increase was noticed in AA, but not in PT. Mackay et al.[13] in their study on pulmonary vessels did not find any significant change in thickness of tunica media with age.

Mario Saldana et al[14] noticed that in the last 3 months of prenatal life, the elastic configuration of the pulmonary trunk and aorta were similar to that observed in newborn. In newborn, elastic fibers were long straight or undulating, parallel and closely arranged, as in the case of the last trimester of intrauterine life. Similar findings were observed in this study. Fragmentation of elastic fibres occurred from the 3^{rd} decade of life in AA and progressed to higher grades by the 6^{th} decade. In contrast, PT showed severe grades of elastic fragmentation from 1st decade of life itself. Similar features were observed by Mario Saldana et al.[14] In the study conducted by L.S. Foster[15] on elastic fibers of aorta, degenerative changes were evident from about 50 years onwards.

Fetal AA and PT showed abundant smooth muscle cells between long straight, compactly packed lamellar elastic fibres. With advancing age, a significant reduction in smooth muscle cells occurred in AA. In contrast, the PT showed an increase in the number of smooth muscle cells in the first decade and underwent slight degeneration thereafter. Smooth muscle attenuation with fatty changes and poorly stained scattered nuclei were noticed in the aorta with advancing age by Foster.[15]

According to Cattell,[16] an increase in collagen concentration occurred in the wall of AA with age over 14–90year range. Quantitative measurement of collagen couldn't be made in our study, but more collagen was observed in tissues with higher grades of elastic fragmentation.

From the 4th decade onwards, tunica adventitia of AA and PT showed a decrease in its proportion. Thinning of

tunica adventitia was detected in studies done by Sankar Dayal Gupta et al.[17]

Thus, in the present study, while a steady increase in the proportion of tunica intima was observed in both AA and PT, the proportion of tunica media was found to increase till the fourth decade and then fall. In all ages, tunica media of pulmonary trunk was thinner when compared to that of AA and elastic fibres suffered high grades of fragmentation from the first decade itself. This can be ascribed to the fall in pulmonary arterial pressure by the end of the first month of extrauterine life, which remains approximately the same throughout the rest of life. Changes in thickness of tunica media of PT between age groups above 50 years and below 50 years were not statistically significant. Tunica adventitia showed a reduction in its proportion from the third decade in both the vessels. However, full wall thickness tended to increase with advancing ages.

The decrease in the proportion of tunica media and adventitia after the fourth and third decade respectively could be possibly due to an increase in elastic fragmentation, collagen fibres occupying the intervals between fragmented elastic fibres, and atrophy and degeneration of smooth muscle cells. These changes in turn lead to stiffness of the vessels. Thus, the cardiac workload gets augmented as higher systolic pressure is required to stretch a stiffer vessel. Moreover, the bolus of blood ejected into the aorta during systole gives rise to a pressure pulse wave, which travels along the wall at a velocity (pulse wave velocity), dependent on its material stiffness. In the stiffer aorta, the pulse wave velocity is higher, so the reflected wave returns soon enough to add to the heart-generated wave during systole, thus increasing systolic pressure and reducing diastolic pressure.

5. Limitations

1. Sex differentiation was not done due to an inadequate number of samples in each group.
2. Quantitative assessment of collagen couldn't be done.
3. Luminal diameter of the vessels was not measured. Hence, a comparison of wall thickness to lumen diameter was not done, which determines the functional stiffness of a vessel.

6. Conclusion

Both AA and PT get stiffer with age. But, after the first decade of life, the degenerative changes in PT are very less when compared to AA. This can be attributed to the fact that pulmonary circulation is a low-pressure system whereas systemic circulation is a high-pressure system. In ascending aorta, the high pressure within the vessel wall, leads to constant stretching of elastic fibres, leading to fragmentation of these fibres, resulting in the transfer of mechanical load to collagen and consequent arterial stiffening seen in aged. The effect of increased stiffness is to reduce the reservoir function of conduit arteries near the heart and to increase pulse wave velocity, both of which increase systolic and pulse pressure.

7. Source of Funding

None.

8. Conflict of Interest

Nil.

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