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Histomorphometric comparison of first and fourth part of vertebral artery in Indian Cadaver

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Abstract
Introduction: The brain receives arterial supply from a pair of internal carotid and a pair of vertebral arteries. Vertebral arteries form vertebrobasilar arterial system and supply the hind brain, as lot of vital functions are attributed to the hindbrain so vertebral artery is one the most important muscular artery in the body. As vertebral artery starts from neck region and has extracranial course and the last part of artery has intracranial course, very less is documented about the histological changes and difference between these two parts. So present study was conducted to understand more of vertebral artery microanatomy.
Aim: The aim of the present study is to compare histomorphometric differences between first and fourth part of vertebral artery.
Materials and Method: vertebral arteries were dissected from thirty embalmed cadavers. Transverse sections were taken from first part near the origin and fourth part from the intracranial course. The annuli were stained and histomorphometric study was conducted using image proplus software. Microanatomy of the both the arteries were studied and compared statistically.
Results and Conclusions: Tunica intima thickness is not significantly different in first and fourth part of vertebral artery. Tunica media thickness decreased in intracranial part compared to the first part of vertebral artery. Internal elastic lamina was observed in most of the specimen and tunica media showed predominance of smooth muscle layers, hence histology is similar to the muscular artery. Cross sectional area of tunica media was compared between first and fourth part of vertebral artery using paired t-test and it is found to be significant.

Keywords: Microanatomy, Vertebral artery, Tunica intima, Histomorphometry, Tunica media

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Introduction
Brain weighs 2.5% of body weight and is a vital organ receiving one sixth of blood flow from heart.(1) The brain receives arterial supply from a pair of internal carotid and a pair of vertebral arteries. The internal carotid arteries supply the frontal, parietal and part of the temporal lobes, while the vertebral arteries through basilar arteries and its terminal branches, supply the occipital and part of temporal lobes together with the brain stem and cerebellum.(2) Many functions are attributed to the hindbrain, its blood supply is crucial and the vertebral arteries are the most important of the medium-sized arteries in the body, supplying the hind brain centers that control cardiac, and respiratory, as well as equilibrium functions.(3) The vertebral artery begins in the root of the neck as the first branch of the subclavian artery on both the sides, traverses the foramina transversaria of the cervical vertebrae and enters the foramen magnum of the skull to meet the vertebral artery of the opposite side at the junction of the pons and medulla oblongata to form basilar artery. The muscular arteries form a low volume, high pressure system and contain smooth muscle fibers in their walls that mediate changes in their compliance. Any such changes, seen as vasoconstriction or vasodilatation may lead to alterations in the blood flow in the artery.(4) As vertebral starts from neck region has extracranial course and the last part of artery has intracranial course, as there can be maximum difference in the histology of the artery at the beginning of the course and at its termination while distributing to the various parts of the brain which is inside the cranium, so first and fourth part of vertebral artery were taken for the study to find major histological differences, as transition occurs gradually and will be maximal at two ends. Reduction in the flow of blood of vertebral artery can lead to ischaemic and neurological deficit. It is dependent on luminal diameter, cross-sectional area and initial size of the artery. Factors like exostoses of cervical vertebrae, mechanical stretching and atherosclerotic plaques can lead to compromise blood flow of vertebral artery. Conductance of streamline blood flow is directly proportional to fourth power of diameter of blood vessel. So present study was conducted to contribute more to the documented data and to help future blood flow studies and for evidence based clinical practice.

Materials and Method
The present study pertains to the histomorphometric study of first and fourth part of vertebral arteries. Vertebral arteries were dissected from thirty embalmed cadavers on both the sides. Each first part of vertebral artery was dissected free of its loose connective tissue attachments from its origin up to the transverse foramen of the sixth cervical vertebra (Fig. 1). The intracranial segment of the vertebral artery

was taken after brain was dissected. Transverse sections were taken from first part near the origin and fourth part from the intracranial course.

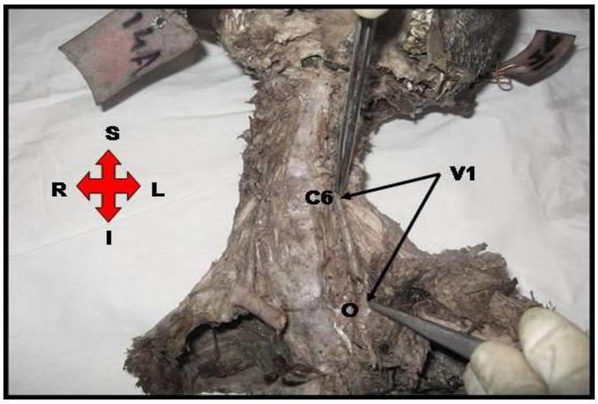

Fig. 1: First part of vertebral artery

Standard H&E staining procedure was done and stained slides were studied under Trinocular Research Microscope and images obtained from it were analyzed on computer with image proplus software. Image-proplus software was calibrated before taking the measurements.

Following histological parameters were measured and recorded:

- D1: inner/internal lumen diameter of the vertebral artery. Measured from one end of luminal surface of endothelial cells of tunica intima to another end of luminal surface of endothelial cells. (Fig. 2)
- D2: perpendicular to D1 measuring inner luminal diameter.
- D3: tunica intima width/thickness measured from luminal endothelial surface to the internal elastic lamina (Fig. 3)
- D4: tunica media width/thickness measured from internal elastic lamina to the external elastic lamina (Fig. 3)
- Di: average of D1 and D2, average inner/internal diameter (D1+D2)/2.
- Do: - outer or external diameter = Di +2D3 +2D4.

csl:- cross-sectional area of lumen = $\pi (Di/2)^2$

csi:- cross-sectional area of tunica intima = $\pi (Di/2+D3)^2$ - csl

csm:- cross-sectional area of tunica media = $\pi (Do/2)^2$ - (csl+csi)

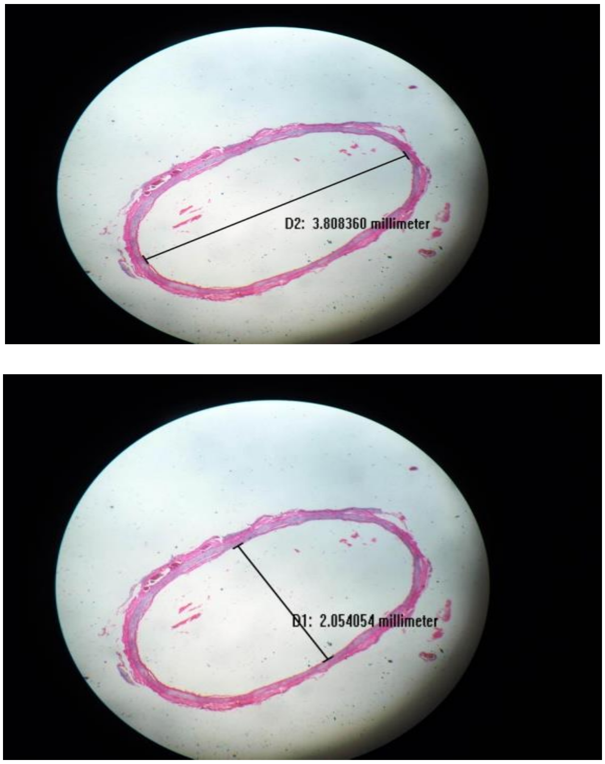

Fig. 2: Illustration showing Vertebral artery (V4 part) 40x magnification measurement of D2, D1 – Inner luminal Diameter

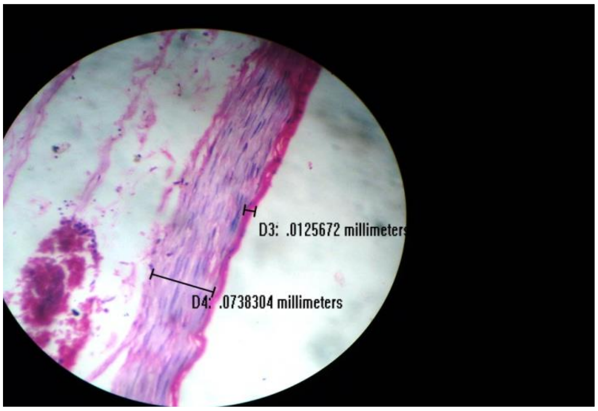

Fig. 3: Illustration showing Vertebral artery (V4 part) 400x magnification D3 – Tunica intima width, D4 – Tunica media Width

The vertebral arteries with incomplete lumen were not taken for the measurement of luminal diameter and cross-sectional area. All measurement were analyzed statistically.

Observation and Results

The specimens obtained from different parts of vertebral arteries for histomorphometric measurements were as follows:

Table 1: No of specimen

	No. of specimen used for measuring luminal diameter	No. of specimens used for tunica intima and media thickness
V1	51	60
V4	56	60
Total	107	120

In the histomorphometric study of the V1 part of the vertebral artery the following results were obtained.

Table 2: First part of the vertebral artery (V1)

	D3	D4	Di	Do	csl	csi	csm
No. of specimens	60	60	51	51	51	51	51
Minimum	0.009 mm.	0.077 mm.	1.774 mm.	2.151 mm.	2.470 mm2	0.060 mm2	0.599 mm^2
Maximum	0.034 mm.	0.311 mm.	3.727 mm.	4.385 mm.	10.911 mm2	0.282 mm2	3.971 mm^2
Mean	0.023 mm.	0.179 mm.	2.444 mm.	2.845 mm.	4.810 mm2	0.155 mm2	1.535 mm^2
SD	0.006	0.047	0.392	0.433	1.640	0.049	0.610

In the histomorphometric study of the V4 part of the vertebral artery the following results were obtained.

Table 3: Fourth part of the vertebral artery (V4)

	D3	D4	Di	Do	csl	csi	csm
No. of specimens	60	60	56	56	56	56	56
Minimum	0.009 mm.	0.047 mm.	1.876 mm.	2.102 mm.	2.764 mm^2	0.084 mm^2	0.349 mm^2
Maximum	0.053 mm.	0.226 mm.	3.692 mm.	4.227 mm.	10.704 mm^2	0.620 mm^2	2.712 mm^2
Mean	0.021 mm.	0.131 mm.	2.494 mm.	2.800 mm.	5.008 mm^2	0.171 mm^2	1.121 mm^2
SD	0.007	0.035	0.401	0.433	1.690	0.085	0.439

Table 4: Paired t-Test between V1-V4 crossectional area of tunica media

Paired parts of vertebral artery	N	p value
V1-V4	51	0.002
P<0.05 is significant		

Discussion

To understand the fluid dynamics, pressure in the arterial wall has to be co related with its wall thickness, the circumferential stress is related to transmural pressure with wall thickness. So microanatomy and histological measurement of vertebral artery will improve understanding of fluid dynamics within the vertebral artery.[5]

Tunica intima width of vertebral artery: Sato et al[(6)] studied vertebral artery in 10 cadavers of Japanese origin in the age group of 8 months to 92 years and studied suboccipital and intracranial part of vertebral artery, his studied showed that tunica intima thickness in suboccipital region was 60 µm and after piercing dura mater the thickness of tunica intima of vertebral artery was 85µm and near the formation of basilar artery was 65 µm. He found no significant difference in the tunica intima width in suboccipital and intracranial region.

In present study tunica intima thickness is not significantly different in first and fourth part of vertebral artery.

Tunica media width of vertebral artery: Sato et al[(6)] measured tunica media width of suboccipital and intracranial part of vertebral artery and found to be 253 µm ± 10 µm in suboccipital region. Intracranially the tunica media width decreased and was 192 µm ± 10 µm.

Similarly Johnson et al[7] studied 18 pairs of vertebral arteries in the cadavers with the age group of 9 months to 86 years, and found no significant difference in tunica media thickness in right and left sided vertebral arteries. The tunica media width in the study was determined at all the levels of vertebral artery. At the origin 0.25mm, near origin 0.21mm, in vertebral region near C4-C5 0.22mm, in the atlanto axial region 0.25mm, in suboccipital region 0.21mm and intracranial region 0.19mm were the mean tunica media thicknesses in the vertebral artery.

Another study conducted by Mitchell et al[(4)] with 94 pairs of vertebral arteries, 40 pairs of suboccipital and 54 pairs of intracranial segments of vertebral arteries were studied in the cadavers of age group 20-80 years. In the study tunica media thickness in the suboccipital region of vertebral arteries were 0.31mm ± 0.09 on the left side and 0.30mm ± 0.11 on the right side. In the intracranial region the tunica media width were 0.21mm ± 0.08 on the left side and 0.20 ± 0.07 on the right side. The difference was significant ($p \leq 0.001$).

Thus in the above 3 studies, it was found that after vertebral artery become intracranial, tunica media thickness significantly decreased.

In the present study, we found that Tunica media thickness decreased in intracranial part compared to the first part of vertebral artery.

Mitchell et al[(4)] studied suboccipital and intracranial part of vertebral arteries and described as muscular artery with a well developed muscular layer in tunica media, prominent internal elastic lamina and discontinuous external elastic lamina seen in most sections.

Microanatomy of first and fourth part of vertebral artery: Carney[(8)] described vertebral artery as muscular artery Wilkinson[(9)] studied vertebral artery in all the parts and described presence of prominent internal elastic lamina. The tunica media was described to be composed of smooth muscle layers with numerous elastic fibrils within. External elastic lamina was also defined.

Author of histology textbooks like Victor[(10)] has described vertebral artery as an example of elastic arteries.

In the present study internal elastic lamina was observed in most of the specimen and tunica media showed predominance of smooth muscle layers, hence histology was similar to the muscular artery as described by Mitchell, Carney and Wilkinson.

To find out the changes in wall of the vertebral artery in different parts, cross sectional area of tunica media was compared between first and fourth part of vertebral artery using paired t-test and it was found to be significant. There were many similarities between present study and older studies although material and method used were different. The present study would help in understanding the relation between the amount of blood flow and wall thickness of the vertebral artery of first and fourth part. As tunica media wall thickness is related to the flow dynamics, this study is important to understand the changes occurring in different parts of artery and pathology occurring in particular part of artery due to various causes like exostoses, mechanical stretching, atherosclerotic plaques etc. leading to relative change in the magnitude of cross-sectional luminal area. This study can be extended further to study pathological changes in particular part of vertebral artery.

Conclusion

- Tunica intima thickness is not significantly different in first and fourth part of vertebral artery.
- Tunica media wall measurement showed difference in thickness was more in first part.
- Internal elastic lamina was observed in most of the specimen and tunica media showed predominance of smooth muscle layers, hence histology is similar to the muscular artery
- Cross sectional area of tunica media was compared between first and fourth part of vertebral artery using paired t-test and it is found to be significant.

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