

Comparative Microscopic Analysis of the Suboccipital and Intracranial Segments of the Vertebral Artery: Structural Variations and Clinical Implications in Indian Cadavers

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ABSTRACT

Introduction: The vertebral arteries are essential for cerebral circulation, supplying critical brain regions including the occipital and temporal lobes, brainstem, and cerebellum. The structural characteristics of the vertebral artery, particularly the suboccipital (V3) and intracranial (V4) segments, influence cerebral blood flow.

Aim: To histomorphometrically analyse the suboccipital (V3) and intracranial (V4) segments of the vertebral artery in Indian cadavers and to understand their structural variations with clinical significance.

Materials and Methods: A descriptive, observational, and cross-sectional study was conducted on 60 vertebral arteries dissected from 34 embalmed cadavers aged 60-75 years, who had died of natural causes. The cadavers were obtained from different regional medical colleges of Gujarat after approval from the Institutional Ethics Committee during the years 2022 to 2024. Segments from V3 and V4 were collected for histomorphometric analysis. Standard histological procedures, including fixation, sectioning, staining with haematoxylin and eosin, and microscopic examination, were performed. The parameters measured included the inner diameter (D1), representing the luminal diameter from endothelial surface to endothelial surface; the perpendicular diameter (D2); the outer diameter (Do); tunica intima thickness (D3); tunica media

thickness (D4); and the cross-sectional areas of the lumen (CSL), tunica intima (CSI), and tunica media (CSM). A paired t-test was used to compare the tunica media cross-sectional areas between the V3 and V4 segments using Statistical Package for the Social Sciences (SPSS) software version 15.0.

Results: Significant differences were observed between the V3 and V4 segments. The tunica media thickness was greater in V3 (0.222 mm) than in V4 (0.131 mm) (p -value <0.001). The inner diameter (Di) was larger in V3 (2.901 mm) compared to V4 (2.494 mm), and the outer diameter (Do) was also greater in V3 (3.407 mm) than in V4 (2.800 mm). The CSL was larger in V3 (6.751 mm²) than in V4 (5.008 mm²), indicating a greater blood-carrying capacity. Histological examination confirmed the vertebral artery to be a muscular artery with a well-developed tunica media and a distinct internal elastic lamina.

Conclusion: This study provides crucial histomorphometric insights into the structure of the vertebral artery, revealing significant regional differences between the V3 and V4 segments. These findings have clinical implications in understanding vertebral artery stenosis, aneurysm formation, and blood flow regulation. Future studies with larger sample sizes and advanced imaging techniques could further elucidate the biomechanical properties and clinical relevance of vertebral artery morphology.

Keywords: Cerebral blood flow, Haemodynamics, Histomorphometry, Tunica media, Vascular anatomy

INTRODUCTION

The brain, although constituting only about 2.5% of total body weight, receives approximately one-sixth of the cardiac output and one-fifth of the body's oxygen at rest [1]. It is highly dependent on continuous blood perfusion for its function, as any significant reduction in blood flow can lead to ischaemia, hypoxia, and potentially cerebral infarction [2]. The vertebral arteries, along with the internal carotid arteries, play a crucial role in supplying blood to the brain, particularly to the occipital and temporal lobes, brainstem, and cerebellum. Each vertebral artery originates from the subclavian artery, ascends through the transverse foramina of the cervical vertebrae, and enters the cranial cavity to form the basilar artery at the pons-medulla junction [3]. The vertebral artery is divided into four parts based on its anatomical course [4]. Blood flow through arteries is influenced by vessel diameter, wall elasticity, and lumen cross-sectional area. The vertebral artery, particularly its suboccipital (V3) and intracranial (V4) segments, plays a significant role in maintaining cerebral blood supply. Pathological conditions such as atherosclerosis, exostoses, or mechanical stretching of these

segments- especially during cervical rotation- can lead to reduced blood flow (Mitchel J, 2003) [5]. According to Poiseuille's law, blood flow is directly proportional to the fourth power of the vessel's radius; hence, even minor changes in diameter can profoundly affect haemodynamic flow [6]. Although asymmetry in vertebral artery size- most commonly with a larger left-sided artery- has been well documented, limited data are available regarding the structural variations between the suboccipital and intracranial segments.

This study aims to fill this gap by histomorphometrically analysing these segments in Indian cadavers, thereby providing valuable insights into their structural characteristics and clinical relevance. Understanding these structural features is essential for evaluating the artery's function and its susceptibility to pathologies such as arterial stenosis, aneurysms, and impaired circulation. The findings of this study hold clinical importance, particularly for surgical interventions such as stenting and dissection repair. Assessing and predicting blood flow dynamics based on these structural parameters can improve our understanding of vertebral artery biomechanics and support the development of more accurate vascular models. Hence,

the objective of this study was to measure the internal and external diameters, tunica intima and tunica media thicknesses, and the cross-sectional lumen area, along with a microanatomical study of these regions. These objectives aim to enhance the understanding of vertebral artery structure and its role in regulating blood flow and ischaemic conditions.

MATERIALS AND METHODS

A descriptive, observational, and cross-sectional study was conducted on 60 vertebral arteries obtained from 34 embalmed cadavers aged 60-75 years, who had died of natural causes. The cadavers were procured from various regional medical colleges of Gujarat after obtaining permission from the Institutional Ethics Committee (IEC No. GMERSMCS/IEC/23/2022). The study was carried out from March 2022 to November 2024.

Inclusion criteria: Normal vertebral arteries with intact walls and preserved cross-sections included in the study.

Exclusion criteria: Arteries damaged during dissection or with disrupted walls were excluded from the study.

Study Procedure

The methodology involved dissection, staining, and morphometric measurement of the vertebral arteries to assess key structural parameters. Dissection was initiated after the students had completed dissections of the face, neck triangles, and deep neck regions. The vertebral arteries were identified at the root of the neck, arising from the subclavian artery. They were dissected from their origin up to the transverse foramen of the sixth cervical vertebra (C6), and then traced through the cervical vertebrae to the posterior atlanto-occipital membrane, as shown in [Table/Fig-1,2]. The intracranial segment was removed following brain excision.



[Table/Fig-1]: Showing dissected part of vertebral artery.



[Table/Fig-2]: Showing V3 part of vertebral artery.

The vertebral artery was classified into four segments:

V1 (Cervical): From its origin to the transverse foramen of C6.

V2 (Vertebral): From C6 to C1.

V3 (Suboccipital): From C1 to the point of dural penetration.

V4 (Intracranial): From dural penetration to the brainstem.

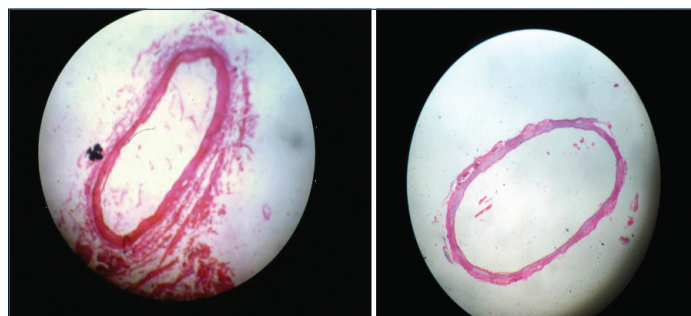
For histomorphometric analysis, 1 cm sections of the vertebral artery were collected from the suboccipital (V3) and intracranial (V4) segments. These samples were labelled and fixed in 10% buffered formalin for two days to prevent autolysis prior to processing. The tissues were then processed overnight using an automatic tissue processor. The processing steps included graded alcohol dehydration, xylene clearing, and embedding in molten paraffin wax.

Microtomy was performed using a rotary microtome, producing sections of 3 μ m thickness. These sections were mounted on glass slides and allowed to dry. Staining was carried out with haematoxylin and eosin following standard protocols, which included dehydration, clearing, staining, and mounting with DPX. Measurements were obtained using a trinocular research microscope equipped with Image-Pro Plus software (version 5.1).

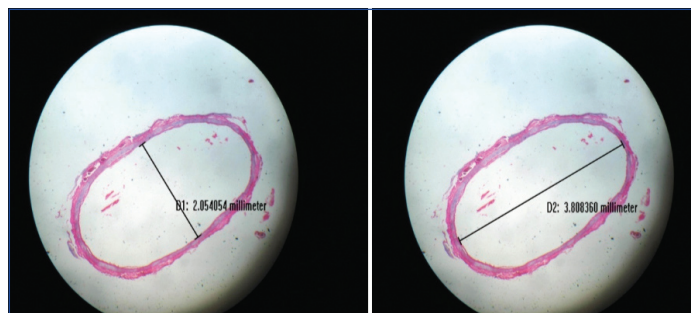
The measured parameters included:

D1 (Inner Diameter): The luminal diameter measured from endothelial surface to endothelial surface.

D2 (Perpendicular Diameter): Measured perpendicular to D1, as shown in [Table/Fig-3,4].



[Table/Fig-3]: Showing vertebral artery V3 and V4 at 40x Magnification (H & E stain).



[Table/Fig-4]: Showing D1 and D2 measurement of V4 part of vertebral artery at 40x Magnification (H & E stain with Image proplus software).

D3 (Tunica Intima Thickness): Measured from the endothelial surface to the internal elastic lamina.

D4 (Tunica Media Thickness): Measured from the internal to the external elastic lamina, as shown in [Table/Fig-5].

The average inner diameter (Di) was calculated as the mean of D1 and D2, while the outer diameter (Do) was determined using the formula:

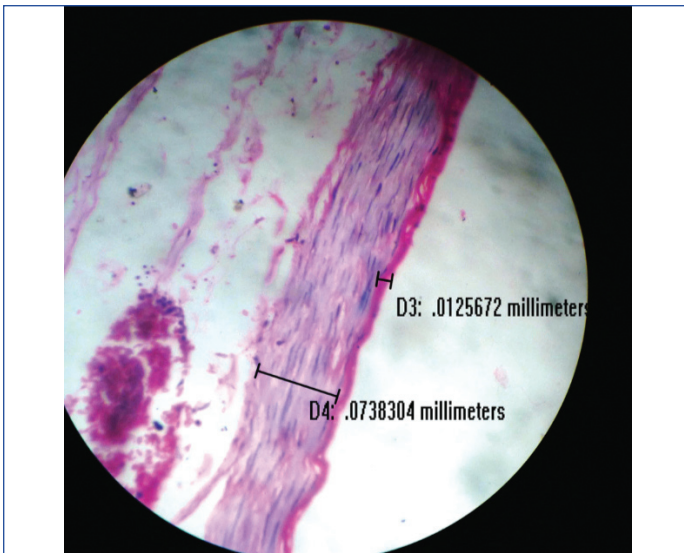
$$Do = Di + 2D3.$$

Cross-sectional areas were computed as follows:

Cross-sectional lumen area (CSL): $\pi (Di/2)^2$

Tunica intima area (CSI): $\pi (Di/2 + D3)^2 - CSL$

Tunica media area (CSM): $\pi (Do/2)^2 - (CSL + CSI)$



[Table/Fig-5]: Showing Vertebral artery (V4 part) 400x magnification D3- Tunica intima width, D4- Tunica media width (H & E stain with Image proplus software).

STATISTICAL ANALYSIS

Statistical analysis was performed to calculate the mean and standard deviation (SD) values. A paired t-test was applied to compare the tunica media cross-sectional areas between the V3 and V4 segments using SPSS software version 15.0.

RESULTS

The findings of the V3 segment, as shown in [Table/Fig-6], and the V4 segment of the vertebral artery, as shown in [Table/Fig-7], revealed notable differences between the two segments, reflecting their distinct anatomical and functional characteristics.

Parameter	No. of specimens	Minimum (mm/mm ²)	Maximum (mm/mm ²)	Mean (mm/mm ²)	SD (mm/mm ²)
D3	60	0.013 mm	0.048 mm	0.024 mm	0.007 mm
D4	60	0.077 mm	0.373 mm	0.222 mm	0.061 mm
Di	60	1.785 mm	4.076 mm	2.901 mm	0.425 mm
Do	60	2.114 mm	4.873 mm	3.407 mm	0.499 mm
CSL	60	2.502 mm ²	13.050 mm ²	6.751 mm ²	1.955 mm ²
CSL	60	0.075 mm ²	0.623 mm ²	0.232 mm ²	0.089 mm ²
CSM	60	0.516 mm ²	4.974 mm ²	2.325 mm ²	0.820 mm ²

[Table/Fig-6]: Showing histomorphometric parameters of V3 (suboccipital) part of vertebral artery.

CSL: Cross-sectional lumen area; CSI: Tunica intima area; CSM: Tunica media area

Parameter	No. of specimens	Minimum (mm/mm ²)	Maximum (mm/mm ²)	Mean (mm/mm ²)	SD (mm/mm ²)
D3	60	0.009 mm	0.053 mm	0.021 mm	0.007 mm
D4	60	0.047 mm	0.226 mm	0.131 mm	0.035 mm
Di	60	1.876 mm	3.692 mm	2.494 mm	0.401 mm
Do	60	2.102 mm	4.227 mm	2.800 mm	0.433 mm
CSL	60	2.764 mm ²	10.704 mm ²	5.008 mm ²	1.690 mm ²
CSL	60	0.084 mm ²	0.620 mm ²	0.171 mm ²	0.085 mm ²
CSM	60	0.349 mm ²	2.712 mm ²	1.121 mm ²	0.439 mm ²

[Table/Fig-7]: Showing histomorphometric parameters of V4 (Intracranial) part of vertebral artery.

In the V3 segment, the tunica intima (D3) demonstrated a mean thickness of 0.024 ± 0.007 mm, while the tunica media (D4) was markedly thicker, with a mean of 0.222 ± 0.061 mm. These measurements indicate a well-developed and structurally reinforced arterial wall, primarily due to the prominent tunica media, which maintains vascular tone and withstands mechanical stress. The average inner diameter (Di) was 2.901 ± 0.425 mm, and the outer diameter (Do) measured 3.407 ± 0.499 mm, indicating a vessel of

relatively large calibre. The CSL averaged 6.751 ± 1.955 mm², while the cross-sectional areas of the tunica intima (CSI) and tunica media (CSM) were 0.232 ± 0.089 mm² and 2.325 ± 0.820 mm², respectively.

These values reflect the structural integrity and functional capacity of the V3 segment, which is subjected to dynamic movement due to its course through the transverse foramina and atlanto-occipital region. The thicker tunica media and broader lumen likely provide mechanical resilience and help maintain stable blood flow during head and neck movements.

In contrast, the V4 segment exhibited comparatively reduced histomorphometric dimensions. The tunica intima had a mean thickness of 0.021 ± 0.007 mm, and the tunica media was thinner than in the V3 segment, with a mean thickness of 0.131 ± 0.035 mm. The inner diameter (Di) measured 2.494 ± 0.401 mm, and the outer diameter (Do) was 2.800 ± 0.433 mm, indicating a relatively narrower vessel. The CSL was 5.008 ± 1.690 mm², while the CSI and CSM were 0.171 ± 0.085 mm² and 1.121 ± 0.439 mm², respectively.

These findings suggest that the V4 segment, being intracranial, has thinner walls and a smaller lumen. Such structural characteristics are consistent with the anatomical constraints and functional demands of the intracranial environment, where the vessel is less exposed to mechanical stress but must maintain uninterrupted cerebral perfusion with minimal resistance.

A direct comparison between the V3 and V4 segments revealed statistically significant differences in tunica media thickness and the cross-sectional areas of both the vessel wall and lumen (p-value <0.05, paired t-test), as shown in [Table/Fig-8]. The V3 segment consistently exhibited higher values across these parameters, confirming that it is anatomically more robust than the V4 segment. This disparity reflects a physiological adaptation of the vertebral artery along its course: the V3 segment is designed to endure external mechanical forces and movement, whereas the V4 segment is optimised for efficient blood flow within the confined and protected intracranial space.

Parameter	V3 (Mean±SD)	V4 (Mean±SD)	p-value
D3	0.024±0.007 mm	0.021±0.007 mm	<0.0001
D4	0.222±0.061 mm	0.131±0.035 mm	<0.0001
Di	2.901±0.425 mm	2.494±0.401 mm	<0.0001
Do	3.407±0.499 mm	2.800±0.433 mm	<0.0001
CSL	6.751±1.955 mm ²	5.008±1.690 mm ²	<0.0001
csi	0.232±0.089 mm ²	0.171±0.085 mm ²	<0.0001
csm	2.325±0.820 mm ²	1.121±0.439 mm ²	<0.0001

[Table/Fig-8]: Showing comparison of V3 and V4 part of vertebral artery with p-value.

DISCUSSION

Blood flow within the arterial circulation is governed by the principles of fluid dynamics, including pressure, resistance, and flow velocity. Understanding these concepts is essential for analysing physiological abnormalities such as arterial obstruction. Histomorphometric analysis of the vertebral artery directly relates to fluid dynamics through parameters such as arterial wall pressure, circumferential wall stress, and vessel dimensions.

Arterial wall pressure results from the interaction between the vessel's elasticity and the volume of blood it contains. A stiffer artery increases resistance, whereas an elastic artery facilitates smooth and continuous blood flow. Circumferential wall stress, defined by Laplace's Law, depends on transmural pressure, internal radius, and wall thickness, and is expressed as $\sigma = P \cdot r / t$, where σ is wall stress, P is transmural pressure, r is internal radius, and t is wall thickness. Thicker arterial walls reduce wall stress, thereby enhancing structural stability [6].

Histomorphometric parameters such as diameter, wall thickness, and lumen area are critical for estimating vascular flow dynamics.

The vessel diameter influences blood flow capacity and resistance, whereas wall thickness and lumen area reflect arterial elasticity and potential blood-carrying capacity.

Comparative studies have shown consistent patterns in vertebral artery structure. Sato et al., reported that tunica intima thickness varied from 60 µm in the suboccipital region to 85 µm after dural penetration [7]. In the present study, the tunica intima thicknesses for the V3 and V4 segments were 0.024 mm and 0.021 mm, respectively, showed statistically significant difference between the two regions or sides. Despite variations in age groups, these findings align with previous research, suggesting structural consistency across populations.

The comparative analysis of tunica media thickness also follows a similar trend. Sato T et al., reported a decrease from 253 µm in the V3 segment to 192 µm in the V4 segment, while Johnson CP et al., and Mitchell documented comparable results [7-9]. The present study recorded tunica media thicknesses of 0.222 mm in V3 and 0.131 mm in V4, confirming a significant reduction from the suboccipital to the intracranial segment. This structural transition likely reflects adaptive modifications as the artery enters the cranium, where it encounters different haemodynamic conditions.

The inner and outer diameters of the vertebral artery also decrease from V3 to V4. Mitchell J reported inner diameters of 3.44 mm in V3 and 2.47 mm in V4, with similar proportional reductions in outer diameters [9]. The present study found inner diameters of 2.901 mm in V3 and 2.494 mm in V4, and outer diameters of 3.407 mm and 2.800 mm, respectively. This reduction in calibre corresponds to a decrease in lumen cross-sectional area, reflecting reduced blood-carrying capacity.

Mitchell J also reported CSL areas of 9.68 mm² in V3 and 5.31 mm² in V4, whereas the present study observed comparatively smaller values, possibly due to differences in the sample population or measurement technique [9]. In a radiological study of the V4 segment, Dharshini P et al., reported that the mean diameter of the vertebral artery at the level of the foramen magnum was 0.32±0.05 cm on the right and 0.322±0.07 cm on the left in females, and 0.30±0.064 cm on the left and 0.26±0.086 cm on the right in males [10].

Histological examination in the present study confirmed the vertebral artery's classification as a muscular artery, consistent with the descriptions of Mitchell J and Carney AL [9,11]. The artery exhibited a well-developed tunica media and a distinct internal elastic lamina. These structural features support its adaptation to withstand varying pressure and flow conditions. However, some authors, such as Victor PE [12], have described the vertebral artery as an example of an elastic artery, indicating that it may exhibit mixed structural features between muscular and elastic arterial types.

A similar histomorphometric comparison of the V1 and V4 segments of the vertebral artery was conducted by Rawal JD et al., [13]. In contrast, the present study compared the V3 and V4 segments, as atheromatous plaques have been reported to occur more frequently at the C1-C2 level of the cervical spine- that is, within the V3 segment- than in the V1 or V2 segments, as demonstrated by Cagnie B et al., [14]. The V3 segment follows a tortuous course before entering the cranial cavity, and this unique anatomical configuration contributes to its distinct structural characteristics compared to the proximal segments.

It was also observed that extracranial dissections of the vertebral artery occur more frequently at the C1-C2 levels of the cervical spine, whereas intracranial dissections are often located near the origin of the posterior inferior cerebellar artery, as reported by Sato T et al., [7]. Smith WS et al., further described that the suboccipital (V3) segment of the vertebral artery is particularly susceptible to dissection, fibromuscular dysplasia, and encroachment by osteophytic spurs within the vertebral foramina [15].

Hence, the present study focused on comparing the suboccipital (V3) and intracranial (V4) segments of the vertebral artery in terms of their dimensions and structural characteristics. This comparison aids in understanding fluid dynamics, the predisposition to atherosclerotic plaque formation, and the higher incidence of extracranial dissection at the V3 level. Additionally, these findings are valuable for surgical and interventional procedures, such as stenting and arterial dissection repair. Furthermore, the insights gained contribute to the development of accurate vascular models for computational fluid dynamics research, thereby advancing both clinical and biomechanical understanding of the vertebral artery.

Limitation(s)

Despite providing valuable insights into the histomorphometric characteristics of the vertebral artery, this study had certain limitations. First, the sample size may not have been large enough to capture the full range of population variability, potentially limiting the generalizability of the findings. Second, factors such as sex and co-morbidities were not extensively analysed, though they could influence arterial dimensions and elasticity. Third, histological processing techniques and measurement variations may have introduced minor inaccuracies in assessing arterial wall thickness and lumen dimensions. Additionally, the study did not account for dynamic physiological changes, such as variations in blood flow related to posture, blood pressure, or cardiac output.

CONCLUSION(S)

The findings clearly demonstrate that the V3 segment of the vertebral artery is anatomically and functionally more robust than the intracranial V4 segment. The V3 segment exhibits a significantly thicker tunica media (≈0.222 mm vs. 0.131 mm in V4), as well as larger lumen and wall cross-sectional areas, reflecting its need to withstand mechanical stress and neck flexion as it traverses the suboccipital region. In contrast, the V4 segment, located within the cranial vault, is characterised by thinner walls and a narrower lumen, features that optimise it for uninterrupted cerebral perfusion under stable intracranial conditions. These morphometric differences are statistically significant (p-value <0.05) and align with existing histomorphometric studies, which note a progressive thinning of vessel layers and reduction in diameter from V3 to V4.

Collectively, these findings underscore a clear functional specialisation:

The V3 segment demonstrates structural reinforcement to accommodate dynamic neck movements, While the V4 segment is adapted for efficient blood flow in a protected, low-stress intracranial environment.

The data carry important clinical implications, particularly for arterial repair and stenting procedures. Despite certain limitations, this study provides a foundational reference for future anatomical and biomechanical research. Further investigations using advanced imaging techniques and physiological modeling are recommended to enhance understanding of vertebral artery biomechanics in both health and disease.

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