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INTRACRANIAL HUMAN VERTEBRAL ARTERY: A HISTOMORPHOLOGICAL STUDY

Rustagi Shaifaly M.[1], Bharihoke V.[2]

[1] Department of Anatomy, V.M.M.C & SJH Delhi, India
[2]Department of Anatomy, U.C.M.S & G.T.B Hospital, Delhi, India

E-mail of Corresponding Author: shaifalyrustagi@yahoo.co.in

ABSTRACT

Cerebrovascular disease due to atherosclerotic involvement of the vertebrobasilar system accounts for one of the major causes of stroke. Intracranial vertebral arteries, one of the feeder vessels of the brain are one of the principal sites implicated. Qualitative and Quantitative histological features of the intracranial vertebral arteries were studied in healthy young adults during routine autopsies.

Thirty pairs of vessels were processed for paraffin sectioning (young adults: 20-40 years). Seven micron thick transverse sections were stained with Hematoxylin and Eosin, Masson's Trichrome and Verhoeff's Stains and observed under the light microscope. The mean luminal diameter was more in left sided vessels whereas tunica intima was thickened more on the right side. Preatherosclerotic ageing changes were observed as early as the third decade of life in apparently healthy young individuals which is an alarming sign for young adults.

Keywords: Artery, Intracranial, Histology, Morphology, Vertebral.

INTRODUCTION

Cerebrovascular disease due to atherosclerotic involvement of the vertebrobasilar system accounts for one third of the cases of stroke. (1) They can be manifested by a wide range of signs and symptoms, including transitory ischemic attacks, ischemic strokes, and chronic insufficiency of cerebral circulation .Symptomatic occlusions of the vertebral artery are most commonly located in the intracranial portion or the fourth part of the vertebral artery. (2)

Our study aims to observe the histological features of the intracranial vertebral artery in apparently healthy adult Indian Population and to study the morphometry of the three tunics and the luminal and histological diameters of the vessels.

MATERIAL AND METHODS

Histological structure of intracranial vertebral arteries was studied in thirty human bodies, 20-40 years of age who had succumbed to road accidents during routine autopsies. Informed consent was obtained from the relatives.

The cranial cavity was exposed and following measurements were taken (in situ):

The length of the intracranial part of the vertebral arteries from the point of entrance of the artery through the foramen magnum to the basilovertebral junction, with a measuring tape.

The intracranial parts were removed and the specimens were preserved in 10% formal saline. 2 mm pieces were taken from the centre of their length and processed for paraffin sectioning. 7µ thick transverse sections were cut on a rotary microtome. Serial sections were stained with Hematoxylin and eosin, Masson's Trichrome and

Verhoeff's Stains. The stained slides were observed under the light microscope.

The following measurements were made with the help of stage and ocular micrometer 4under 3.2x (3):

1) Measurement of the luminal diameter: The measurements were taken along the maximum and minimum diameter of the vessels. The mean of the two readings was taken.

The following measurements were made under 40x:

1) The tunica intima, from the endothelium to internal elastic lamina

2) Tunica media, from internal elastic lamina to junction of media and externa

3) Tunica adventitia, from junction of media and adventitia to periphery

Measurements of external diameters: The external diameter of the vessels in histological sections was calculated using the formula: [luminal diameter / 2 + (thickness of tunica intima + tunica media + tunica adventitia) x 2].

The external diameter calculated histologically was compared to the gross external diameter and shrinkage factor was calculated. Five measurements were taken at random from every fifth section and their mean calculated. The thickness of the three layers was compared on the right and left side and were statistically evaluated.

RESULTS

The vertebral arteries exhibited three well defined layers, tunica intima, tunica media and tunica adventitia. The subendothelial connective tissue showed thickened areas in most of the cases.(Fig 1) Focal fibroelastic masses(fig 2) in excess of one fifth the thickness of wall were seen in three cases. At these focal areas of intimal thickening, the connective tissue beneath the endothelial cells was more loosely arranged as compared to the deeper part of tunica intima. The deeper part of the tunica intima was rich in longitudinally running smooth muscle cells, interspersed in between elastic fibres.

The internal elastic lamina stood out as a prominent waxy layer of elastic fibres. Focal areas of reduplication (fig 3) of the internal elastic lamina were seen in seven cases. (Table 1)

The tunica intima varied from very thin to one fifth of thickness of the wall at various sites.

Case studies

In a 28 year old male who died due to right parietal hematoma, both the exhibited arteries exhibited thickenings in the intima with reduplications at 1 site in all the sections in right vertebral artery and 0-1 site in the left vertebral artery .(fig 3)

In a 38-year-old male who died due to fracture of the anterior cranial fossa. A thickened area (Fig 1) is seen in the intima of the left vertebral artery at one site in four sections. The internal elastic lamina is seen as a single prominent layer in these sections. This thickened area is covered by endothelial cells; it is composed of sparse elastic fibres running in the superficial part of this area with empty looking spaces. In the deeper part longitudinally cut muscle cells more compactly arranged are seen. The media is seen to be made up of circularly arranged smooth muscle cells and intervening connective tissue fibers. The right vertebral artery is found to be hypoplastic (Fig 2) with no such areas in the tunica intima.. The remains of the external elastic lamina are found in all the sections in both the vertebral arteries in this individual.

Mean thickness of the three tunics

The mean thickness of tunica intima of the vertebral artery on the right side 19.93 ±8.47µ and on the left side = 17.07± 6.93 µ (p= 0.09)

The mean thickness of the tunica media of vertebral artery on the right side 101±24.53 µ and on the left side 108.93±29.05 µ (p= 0.19)

The mean thickness of tunica adventitia in vertebral artery on right side is 58.2±11.59 µ and on left side = 62.47±12.24 (p= 0.13)

The tunica media comprises of circumferentially arranged smooth muscle cells. In the vertebral arteries exclusive circumferential arrangement is seen .The smooth muscles are arranged in 16-22 lamellae in vertebral artery.

In most of the cases, the external elastic lamina cannot be defined, it is seen as fragmented wavy elastic fibres in five cases in the right vertebral artery and three in the left vertebral artery.

The **mean luminal diameter** of the vertebral artery on the right side is 2.175±0.27mm and on the left side is 2.20±0.211mm (p=0.13)

The **mean histological outer diameter** of the vertebral artery on the right side is 2.46±0.42mm and on the left side is 2.54±0.3mm. (p=0.22)

DISCUSSION

It is well documented that in muscular arteries the endothelium rests directly on the internal elastic lamina. (4).In the present study it is seen that variably thickened subendothelial tissue is found in apparently healthy young individuals.

Wilkinson studied the extracranial and intracranial structure of twenty vertebral arteries between the ages of 60 and 75 from causes that were neither vascular nor neurological (5). In the intradural segment he found

a) adventitial collagen fibres were less marked and the external elastic lamina was either absent completely or represented by sparse single elastic fibrils only.

b) The width of the medial coat was noticeably thinner in this part of the artery than extradurally**.**

Fibroelastic masses in the subendothelial tissue were seen in basilar arteries in nine of the thirty six cases .Blumenthal et al (6) and Tuthill (7) documented these in six of the twenty cases he studied. Similar findings in the present study suggest that these fibroelastic masses begin to form in even the younger age groups. Substantial increase in the elastic fiber in the intima found in patients along with the migration of smooth muscle cells are distinct ageing changes in the vessels documented by Simionescu N and Simionescu M (8).

Splits in the internal elastic lamina have been observed in seven cases in our study. Similar areas of splitting with fibroelastic masses in between the split fibers in the large cerebral arteries were documented by Blumenthal et al, Tuthill and Hassler (9). However their studies were on children and at branching points of vessels. They considered these to be physiological. In the present study care has been taken to avoid branching points. The presence of split connective tissue fibers and circularly running smooth muscle cells in between these reduplications suggest that they are early ageing changes.

Turliuk et al (10) documented arterial intima thickness to be 68.4 +/- 6.3 micron in the third part of vertebral artery. They also observed a moderate increase in the arterial wall thickness on the left (485.15 +/- 35.35 micron) as compared with that of the right VA (416.25 +/- 1l3.42 micron) (P = 0.12), at the expense of the middle tunic and adventitia. However our study is on the fourth part or the intradural part and considerable differences exist between these two parts of the vessel.

Jovanikivic et al (11)measured intimomedial thickness (IMT) of the vertebral arteries first part IMT = 0.585 ± 0.134 mm in 50 individuals and second part IMT = 0.782 ± 0.248 mm and hypothesized the IMT to be an atherosclerosis indicator.

Keith et al (12) reported histological changes in the tunica intima and tunica media of eighteen dogs using histological staining techniques. Fifteen dogs had abnormalities of tunica intima or tunica media in at least one arterial section examined. Of all arterial sections examined, 40% had histological changes like loss of smooth muscle cells and elastin of the tunica media and replacement by collagen. We have observed abnormalities in the tunica intima of most of the

vessels and in 7 of the 30 cases (23.3%) splits of the internal elastic lamella have been found.

Moossy (13, 14) also found left sided intracranial vertebral arteries to be more involved in atherosclerotic lesions. In accordance with our study he also observed arteries of patients below 39 years of age to be free of thrombus formation.

Johnson et al(15) stained sections from 34 vertebral arteries and reported collagen counts were higher and elastic counts substantially lower within the intracranial segment. They reported degenerative changes were often focal and spared the intracranial segment almost completely.

The external elastic lamina cannot be defined, it is seen as fragmented wavy elastic fibres in five cases in the right vertebral artery and three in the left vertebral artery. Blumenthal et al found fine elastic filaments in 16 cases in the place of the external elastic lamina. Ratinov (16) found it in a few cases in the intracranial intracavernous part of carotid artery as an indistinct lamina. Wilkinson observed that it disappeared completely 1cm distal to the point of dural attachment in the vertebral artery. These findings suggest that the external elastic lamina is not a well defined layer in the intracranial vessels.

The mean histological outer diameter of the vertebral artery on the right side is 2.46±0.42mm and on the left side is 2.54±0.3mm. . Mitchell and McKay (17) found the microscopic outer diameter of the vertebral artery to measure 2.04±0.55mm on the left side and 2.03±0.52mm on the right side in formalin fixed cadavers. The slightly lower readings in their study are because they did not include the tunica adventitia in calculating the outer diameter. Moreover their study included 45 Blacks and 13 Whites.

In the eight white females in their study they recorded the outer diameter 2.13±0.34/mm on the right side and 2.42±0.39mm on the left side which were significantly more in size. They concluded that the females in the white ethnic group may be more at risk as regards vascular accidents after cervical spine manipulation. Based on his conclusion in the present study we can presume that the larger left side vertebral arteries recorded could make the Indian population more at risk of ischemic events.

The mean luminal diameter of the vertebral artery on the right side is 2.10 ±0.38 mm and on the left side is 2.16±0.25mm. Mitchell and McKay (17) recorded it to be 1.73 ±0.51mm on the right side and 1.74 ±0.50mm on the left side. The slightly lower readings in their study could be because of the different population they studied.

CONCLUSION

We have observed changes in the tunica intima of 7 of the 30 cases (23.3%).We would like to summarize that the Preatherosclerotic changes observed in the tunica intima of the vertebral arteries is an alarming sign for the young population and indicates towards the lifestyle changes that are imperative for our blood vessels.

The mean luminal diameter of the vertebral artery on the right side is 2.10 ±0.38 mm and on the left side is 2.16±0.25mm. The larger left side vertebral arteries recorded could make the Indian population more at risk of ischemic accidents especially after cervical spine manipulation.

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CONFLICT OF INTEREST

None declared.

FIGURES

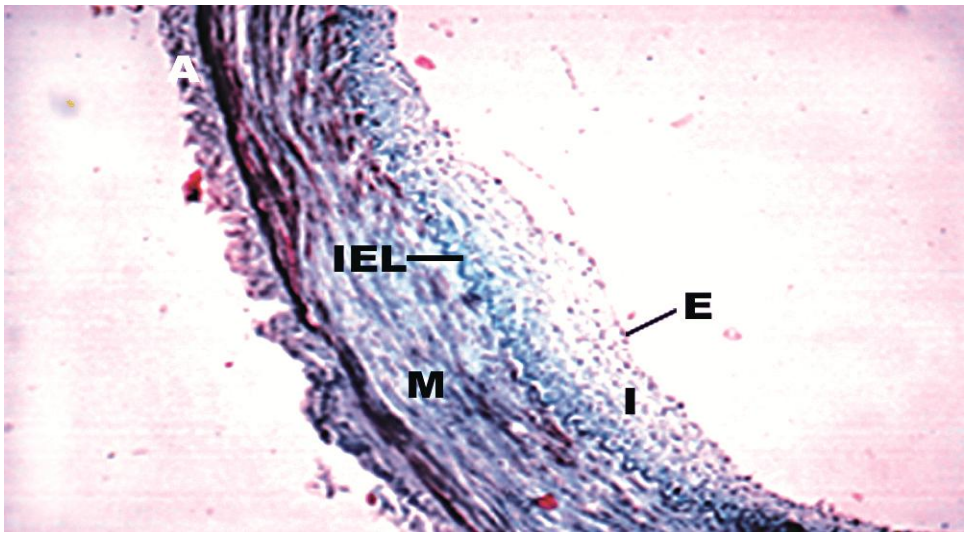

Fig. 1: Photomicrograph of transverse section of left vertebral artery of 38 year old male showing thickened area in intima covered with endothelium, although the internal elastic lamina is not split. Internal elastic lamina (IEL), Endothelium (E), Tunica intima (I), Tunica media (M), Masson's Trichrome Stain, counterstained with Aniline blue (x100)

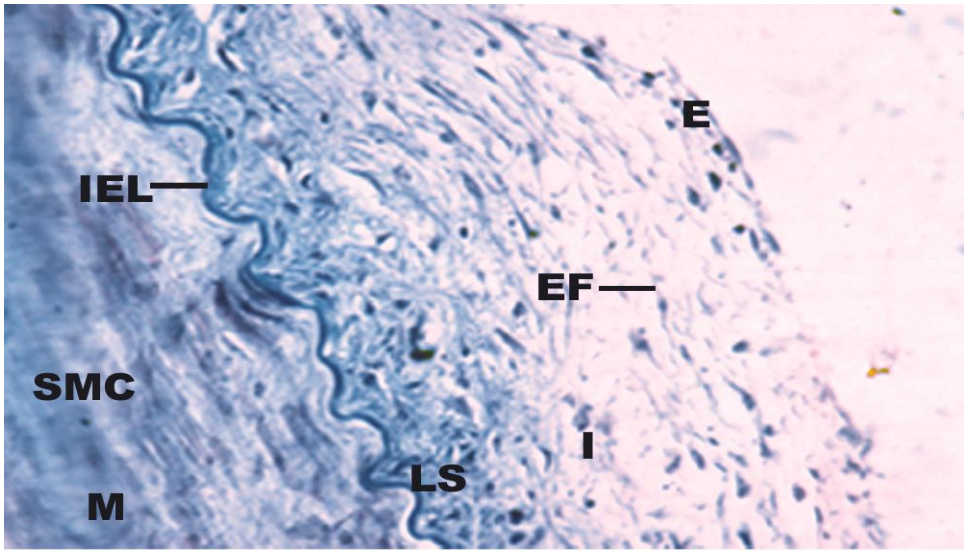

Fig. 2: Photomicrograph of transverse section of left vertebral artery of 38 year old male showing sparsely arranged fibres in superficial part and densely arranged smooth muscle cells in the deeper part of tunica intima.

Internal elastic lamina (IEL), Endothelium (E), Elastic fibres (EF), Longitudinally cut smooth muscle cells (LS), Tunica intima (I), Tunica media (M), Smooth muscle cells (SMC) Masson's Trichrome Stain counter stained with Aniline blue (x1000)

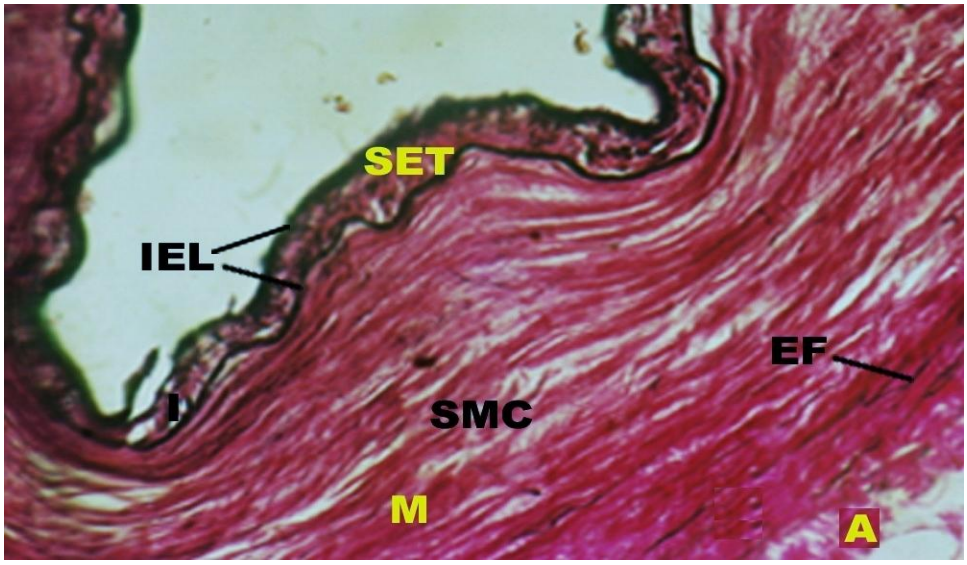

Fig. 3: Photomicrograph of transverse section of right vertebral artery of 28 year old male showing the variably thickened subendothelial tissue (SET), Internal elastic lamina (IEL) is split at different places. Circularly arranged smooth muscle cells (SMC) seen in the tunica media (M).
Tunica intima (I), Tunica adventitia (A), Collagen fibres (CF), Verhoeff's stain (x100)

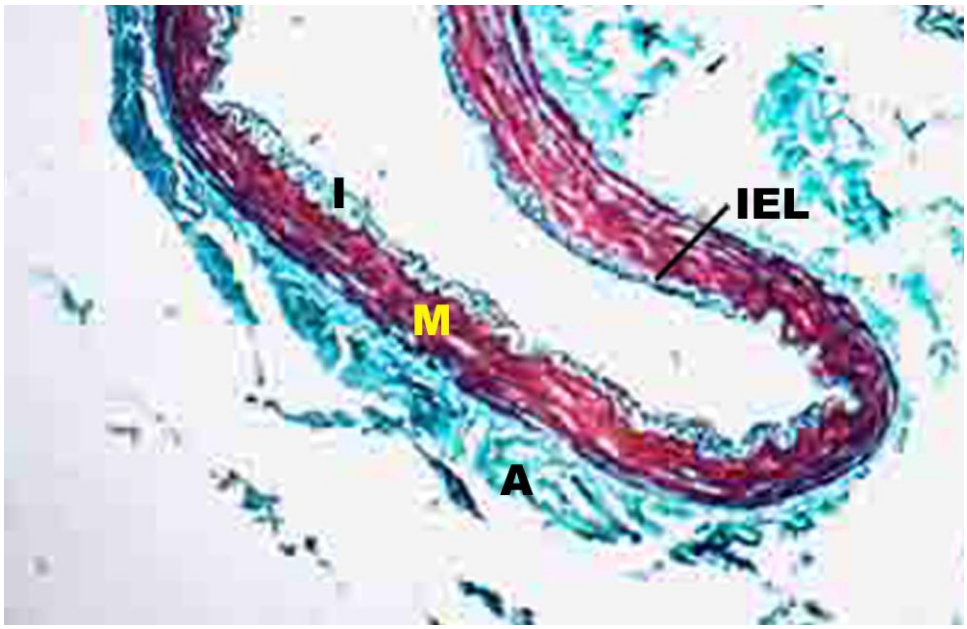

Fig. 4: Photomicrograph of transverse section of hypoplastic right vertebral artery of 38 year old male showing uniform subendothelial layer.
Internal elastic lamina (IEL), Tunica intima (I), Tunica media (M), Tunica adventitia (A), Masson's Trichrome Stain, counterstained with light green (x100)

Table 1 : **Number of sites of reduplication of the internal elastic lamina**

Age	Right vertebral artery	Left vertebral artery
28	1 site in all sections (2 layers)	0-1 site (2 layers)
35	0-1 site (2 layers)	-
34	0-1 site	-
36	-	1 site in all sections
26	0-1 site (2 layers)	-
35	0-1 site (2 layers)	-
28	0-2 sites (2 layers)	-

Table 2: Outer diameter, luminal diameter, thickness of tunica intima, thickness of tunica media and thickness of tunica adventitia of vertebral arteries in 30 individuals

S. No.	VAHOD R (mm)	VALU R (mm)	VAI R (µ)	VAM R (µ)	VAAR (µ)	VAHOD L (mm)	VALU L (mm)	VAI L (µ)	VAM L (µ)	VAA L (µ)
1.	2.21	1.70	34	158	62	1.85	1.46	30	110	56
2.	0.98	0.73	17	62	45	2.06	1.78	10	82	50
3.	2.36	1.95	21	98	88	2.48	2.18	14	90	45
4.	2.84	2.45	22	110	61	2.76	2.42	17	98	54
5.	2.55	2.22	12	94	60	2.67	2.35	23	82	54
6.	2.41	1.90	38	154	63	2.30	2.05	9	75	40
7.	2.19	1.90	15	84	48	2.18	1.90	12	80	50
8.	1.66	1.40	21	68	40	2.15	1.88	8	77	52
9.	2.38	2.01	34	94	55	2.28	1.94	23	96	50
10.	2.36	2.09	14	75	46	2.48	2.04	30	120	72
11.	2.88	2.42	24	145	60	2.93	2.54	15	115	67
12.	2.28	2.04	10	68	40	2.38	2.05	13	98	54
13.	2.86	2.55	19	84	52	2.88	2.48	20	140	40
14.	2.57	2.25	8	94	60	2.57	2.22	15	100	62
15.	2.87	2.46	23	110	70	2.88	2.48	10	120	70
16.	2.58	2.25	30	87	50	2.54	2.10	18	122	82
17.	2.77	2.40	32	98	55	2.85	2.38	28	138	69
18.	2.08	1.78	14	80	55	2.12	1.80	12	89	60
19.	2.15	1.85	20	90	40	2.35	2.05	9	84	56
20.	2.38	2.04	11	100	60	2.55	2.16	20	110	67
21.	2.66	2.25	15	118	70	2.49	2.11	12	108	70
22.	2.16	1.85	12	90	55	2.57	2.16	20	110	75
23.	2.74	2.30	29	121	70	3.08	2.45	26	229	60
24.	2.88	2.45	14	130	72	2.66	2.26	10	115	75
25.	2.97	2.50	29	127	80	3.17	2.70	24	139	70
26.	2.48	2.13	26	88	60	2.57	2.16	29	98	80
27.	2.36	2.08	9	81	50	2.45	2.10	12	100	65
28.	2.58	2.25	22	92	49	2.68	2.32	15	102	65
29.	2.78	2.42	12	110	60	2.56	2.15	9	118	79
30.	2.88	2.48	11	120	70	2.61	2.16	19	123	85
Mean	**2.46 ±0.42**	**2.10 ±0.38**	**19.93 ±8.47**	**101.03 ±24.53**	**58.20 ±11.59**	**2.54 ±0.30**	**2.16 ±0.25**	**17.07 ±6.93**	**108.93 ±29.05**	**62.47 ±12.24**

VAHODR= Vertebral artery histological outer diameter right side; VALUR=Vertebral artery luminal diameter right side; VAIR= of carotid artery intima right side; VAMR= Vertebral of carotid artery medial right side; VAAR= Vertebral of carotid artery adventitia right side; VAHODL=Vertebral artery histological outer diameter left side; VALUL=Vertebral of carotid artery intima media left side; VAIL= Vertebral of carotid artery intima left side; VAML= Vertebral of carotid artery media left side; VAAL= Vertebral of carotid artery adventitia left side

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