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ORIGINAL ARTICLE

Histomorphometric study of intracranial internal carotid artery in man

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Summary

The incidence of cerebrovascular diseases in man is on an increase. Atherosclerotic lesions in intracranial vessels are the usual cause. The normal parameters and histological changes in the intracranial internal carotid arteries, one of the feeder vessels to the brain, were studied in apparently healthy young adults of the Indian population.

Gross and histomorphometric features of the intracavernous part of the internal carotid artery (ICA) were studied in the Indian population, in order to determine normal parameters, and observe structural changes in healthy young individuals. Length and outer diameter of ICA were taken in 100 autopsy cases. Cases were grouped according to age – Group I: <20 years, Group II: 20-40 years, and Group III: >40 years. Thirty pairs of vessels were processed for paraffin sectioning (young adults: 20-40 years). Seven-micrometers-thick transverse sections were stained with Hematoxylin and Eosin, Masson's Trichrome and Verhoeff's Stains.

The length was found to be significantly greater on the left side in Groups II and III. The outer diameter was found to be significantly longer in the left side Group III. The mean thickness of *tunica intima* was found to be greater on the right side.

Preatherosclerotic ageing changes were observed in the third decade of life in apparently healthy young individuals.

Key words: Atherosclerosis – Cerebral vessels – Indian population – Internal carotid artery

Introduction

The arteries of the body are composed of three concentric layers, the *tunica intima*, *tunica media* and *tunica adventitia* (Gabella, 1995). The proportion of these three layers changes with the size of the vessel. The individual constituents of the vascular wall show constant changes with age. The most affected arteries are the aorta, coronary and cerebral. It is claimed that one is as old as one's arteries.

The age-related changes have been described in detail in large elastic arteries like the aorta. The intracranial arteries are muscular arteries which differ from their extracranial counterparts in having thin walls and proportionately more elastic fibers in the *tunica media* (Leeson et al., 1985).

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The internal carotid arteries are of great significance, because they are one of the feeder vessels to the brain. They have been observed to be the initial sites of intracranial atherosclerosis (Moossy, 1966). Cerebrovascular accidents due to atherosclerotic stenosis or thrombotic occlusion in these vessels are a common occurrence. Histological changes in the microscopic structure of these vessels in apparently healthy young adults have not been studied in detail.

The incidence of cerebrovascular diseases in man is on an increase. This could be due to lifestyle changes. The advances in investigative procedures and surgical treatment make it imperative for us to know about the normal parameters of these vessels in the Indian population.

Materials and Methods

The intracavernous part of the internal carotid arteries was studied in human bodies during routine autopsies carried out in the Department of Forensic Medicine, University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi. Arteries from 100 bodies of both sexes and all age groups were dissected.

The cases were grouped according to age as follows:

Group I: Less than 20 years

Group II: 20 to 40 years

Group III: More than 40 years.

The scalp was incised and the vault of the skull was opened with a bone saw. Then the *dura* was incised, and the brain was removed after cutting the nerves. The internal carotid arteries were exposed by dissecting the cavernous sinus. The following measurements were taken:

a) The length of the cavernous part of the internal carotid artery from entry of the vessel through foramen lacerum to the site of exit through the cavernous sinus (with a measuring tape).

b) The external diameter at the midline of the vessel (with a vernier caliper).

The intracavernous part of the arteries was removed from the cranial cavity of thirty cadavers, aged between 20 and 40, who had succumbed to road accidents. The specimens were preserved in 10% formal saline. Two mm pieces were taken from the centre of their length and processed for paraffin sectioning. Seven-μm-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 taken with the help of stage and ocular micrometer under 3.2x:

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) The *tunica media*, from internal elastic lamina to junction of *media* and *externa*.

3) The *tunica adventitia*, from junction of *media* and *adventitia* to periphery.

The measurements were taken at random from every fifth section, and their mean calculated. The measurements were statistically evaluated.

Results

Gross anatomy

The internal carotid arteries were seen running medially in their course from where they emerge in the *foramen lacerum* to the site where they pierce the *dura mater* lateral to anterior clinoid process. They were fairly uniform in thickness. They were filled with blood and no columns of obstruction could be seen.

The vessels were related to the side of the body of the sphenoid. Some vessels were straight in their course, and some showed various degrees of curves, which were called 'tortuosity' of the vessels in this study. The various patterns of tortuosity are shown in Fig. 1.

In the present study, the internal carotid arteries in their intracavernous part were found to be curved with a medial convexity on both sides in 24% of cases, lateral convexity on both sides in 5% of cases, medial convexity on the right side and lateral convexity on the left in 5% of cases, and lateral convexity on the right side and medial convexity on the left side in 4% of cases. Straight on the right side and medial convexity in the left sided arteries in 11% of cases, straight on the right side and lateral convexity in the left-sided arteries in 4% of cases, straight on the left side and medial convexity in right-sided arteries in 7% of cases, straight in the left side and lateral convexity in right-sided arteries in 2%, and uniformly straight in their course in 38% of cases.

The arteries were apparently normal in texture in 99 cases. The arteries were filled with blood, and all vessels seen in this study were uniformly red in colour. There was no evidence of discoloration on the surface of any artery. In one 65-year-old male, the vessels were comparatively dilated and gritty. Death in this individual was due to suspected poisoning. All organs were congested on postmortem examination.

The gross parameters measured are given in Tables 1 and 2. The length was found to be significantly greater on the left side in Group II ($p=0.013$) and Group III ($p=0.002$). In Group III the outer diameter of the vessels on the left side is significantly longer than on the right side ($p=0.018$).

Table 1. Mean length of the internal carotid artery.

	Right (mm)	Left (mm)
Group I	22.67±2.31	23.33±2.08
Group II	21.12±2.46	21.57±2.46
Group III	21.09±2.15	21.70±2.24

Table 2. Mean outer diameter of the internal carotid artery.

	Right (mm)	Left (mm)
Group I	3.5±0.3	3.56±0.23
Group II	4.19±0.45	4.22±0.46
Group III	5.03±0.49	5.12±0.57

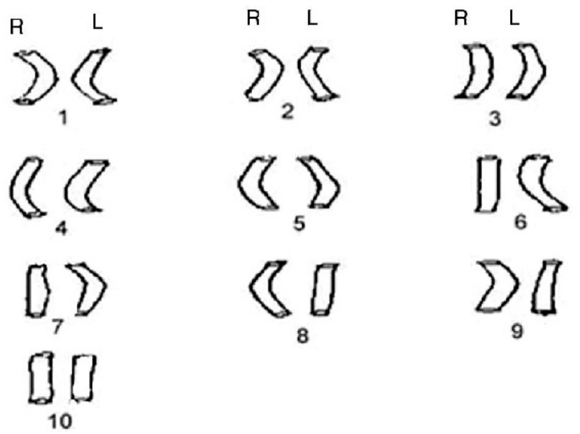

Fig. 1. Patterns of tortuosity of the internal carotid arteries in 100 cases: (1) 14%, (2) 10%, (3) 5%, (4) 4%, (5) 5%, (6) 11%, (7) 4%, (8) 2%, (9) 7%, (10) 38%.

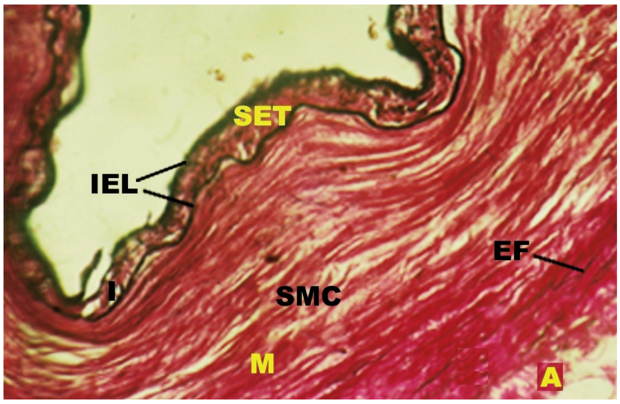

Fig. 2. Transverse section of left carotid artery of 36 year-old male showing reduplication of internal elastic lamina (IEL) and intervening subendothelial tissue (SET), fine elastic fibres (EF) are seen at the junction of *tunica media* (M) and *tunica adventitia* (A), Smooth muscle cells (SMC), *tunica intima* (I). Verhoeff's stain (x 400).

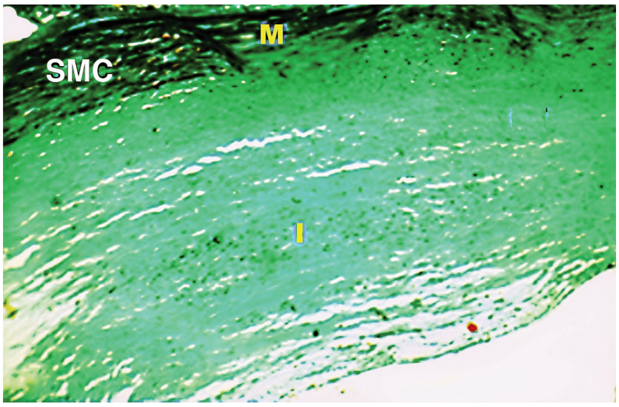

Fig. 3. Transverse section of right carotid artery of 28 year-old male with grossly thickened *tunica intima* (I), smooth muscle cells (SMC) in the *tunica media* are seen. Tunica media (M), Masson's Trichrome, counterstained with light green (x 100).

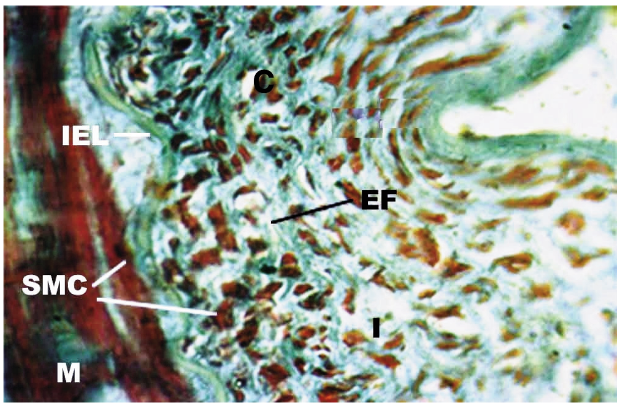

Fig. 4. Transverse section of right carotid artery of 28 year-old male showing longitudinally arranged smooth muscle cells (SMC) in the deeper area of thickened *tunica intima*, numerous capillaries (C) seen in subendothelial tissue, the smooth muscle cells are oriented more circularly in the superficial part to *tunica intima* (I). Internal elastic lamina (IEL), Elastic fibres (EF), *Tunica media* (M), Masson's trichrome stain, counterstained with light green (x 1000).

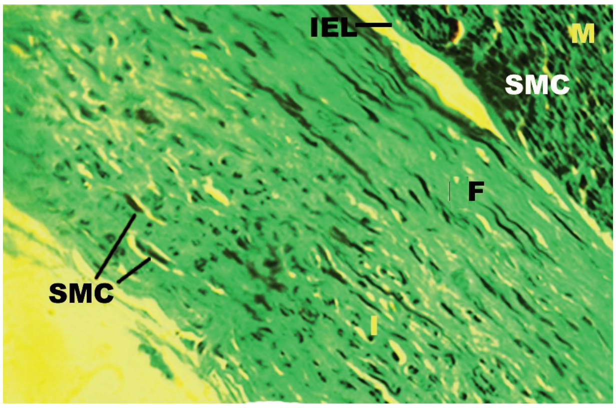

Fig. 5. Transverse section of right carotid artery of 28 year-old male showing circularly arranged smooth muscle cells in deeper part of *tunica intima* and obliquely cut smooth muscle cells in tunica media. Internal elastic lamina (IEL), Smooth muscle cells (SMC), *Tunica intima* (I), *Tunica media* (M), Connective tissue fires (F) Masson's Trichrome Stain, counterstained with light green (x 400).

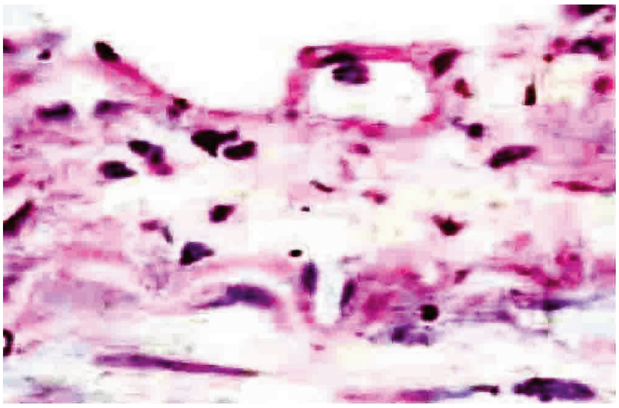

Fig. 6. Transverse section of right carotid artery of 28 year-old male under high magnification showing capillaries in subendothelial tissue. Haematoxylin and eosin stain (x 1000).

Microscopic anatomy

The internal carotid arteries exhibited free well-defined layers, *tunica intima*, *tunica media*, and *tunica adventitia*. The subendothelial connective tissue showed thickened areas in most of the cases (Fig. 2). Focal fibroelastic masses in excess of one fifth the thickness of wall were seen in three cases (Fig. 3). At these focal areas of internal thickening, the connective tissue beneath the endothelial cells was more loosely arranged when compared with the deeper part of *tunica intima*. The deeper part of the *tunica intima* was rich in longitudinally-running smooth muscle cells, interspersed with elastic fibres (Fig. 4).

The internal elastic lamina stood out as a prominent waxy layer of elastic fibres. Focal areas of reduplication of the internal elastic lamina (Fig. 2) were seen in twelve cases. The distribution of the splits is shown in Table 3. Multiple splitting of the internal elastic lamina was seen in three cases in the right carotid artery. The *tunica intima* varied from very thin to one fifth of thickness of the wall at place (Fig. 3). An interesting finding is the presence of numerous capillaries in these areas of thickened subendothelial tissue in the *tunica intima* (Fig. 6).

The mean thickness of the *tunica intima* of the carotid artery on the right side is 26.27±12.71 µm, and the mean thickness of the *tunica intima* of the carotid artery on the left side 25.23±9.59 µm (Table 4). The average thickness of the *tunica media* of the carotid artery on the right side is 196.93±19.76 µm, and on the left side 198.73±17.19 µm (Table 4).

Table 3. Number of sites of reduplications of the internal elastic lamina.

Age	Right	Left
28	1-3 sites (>5 layers)	0-1 site (2-3 layers)
38	0-2 sites (2-3 layers)	
35	2 sites in all sections (2 layers)	0-3 sites (2 layers)
29	0-1 site (2 layers)	
34	0-2 sites (2-3 layers)	1 site in all sections (2 layers)
36		0-2 sites (2-3layers)
38	2 sites in all sections (2 layers)	1-2 sites (2 layers)
39	1 site in all sections (2 layers)	
38	1-3 sites (>5 layers)	1-2 sites (2-3 layers)
32		0-1 site (2 layers)
35		0-2 sites (2layers)
28	0-3 sites (>5 layers)	1-2 sites (2-3 layers)

Table 4. The mean thickness of three layers.

	Right (µm)	Left (µm)
Tunica intima	26.27±12.71	25.23±9.51
Tunica media	196.93±19.76	198.73±17.19
Tunica adventitia	73.10±12.99	71.17±11.12

The *tunica media* comprised circumferentially arranged smooth muscles arranged in 28-32 lamellae. The external elastic lamina was not well defined, seen as fragmented wavy elastic fibres between the *tunica media* and *adventitia* in six cases in the right carotid artery. The *tunica adventitia* was composed of longitudinally arranged connective tissue fibers consisting of collagen fibers, fibroblasts and *vasa vasorum*. In three instances in the right carotid artery it is arranged as two layers of inner, longitudinal and outer collagenous fibres, and in two cases in the left. The *tunica adventitia* merges with the surrounding connective tissue.

The mean thickness of tunica adventitia in carotid artery on right side is 73.10±12.99

µm and the mean thickness of tunica adventitia in carotid artery on left side = 71.17±11.12 µm (Table 4).

The mean luminal diameter of the carotid artery on the right side is 3.37±0.38 mm and on the left side is 3.72±0.41 mm (Table 5).

Case studies

In a 28-year-old male who died due to right parietal hematoma, reduplications were observed in 1-3 sites in the right carotid artery, and from 0-1 site in the left carotid artery. In this individual, the right carotid artery exhibited one site (Fig. 3) where the *intima* was grossly thickened in one-third the circumference of the vessel in all the sections observed. The numerous thin elastic fibrils were seen in the superficial part of this thickened area, and in the deeper part smooth muscle cells interspersed with elastic fibres are seen. The *tunica media* is thinned out at one site over the thickened *tunica intima*. At other sites the *tunica media* is composed of circularly arranged regularly running smooth muscle cells. The *tunica externa* is seen as a layer of longitudinally running collagenous fibres in all the sections.

In a 28-year-old male, 1-3 sites of reduplication are observed in the right carotid artery

Table 5. Outer diameter, luminal diameter, thickness of *tunica intima*, thickness of *tunica media* and thickness of *tunica adventitia* of carotid arteries in 30 individuals.

S. No.	CALUR (mm)	CAIR (µm)	CAMR (µm)	CAAR (µm)	CALUL (mm)	CAIL (µm)	CAML (µm)	CAAL (µm)
1	3.72	70	224	112	3.95	55	230	110
2	3.88	46	206	82	3.84	34	196	70
3	4.10	48	230	70	3.62	28	227	60
4	3.30	25	180	60	3.25	15	176	66
5	3.16	21	182	72	3.10	24	184	60
6	4.18	15	210	63	4.22	21	202	74
7	3.15	30	190	90	2.97	16	178	60
8	3.62	24	182	68	3.67	28	206	70
9	4.10	39	227	60	3.82	20	210	60
10	3.70	18	224	94	3.90	28	190	72
11	4.35	25	229	80	4.55	21	232	50
12	4.00	32	192	60	3.95	38	206	63
13	3.40	14	170	58	3.26	10	188	56
14	4.05	24	185	70	4.15	25	204	68
15	3.30	22	170	80	3.44	20	178	70
16	3.70	30	185	65	3.60	29	190	65
17	3.86	35	198	80	3.92	38	208	82
18	3.94	24	212	84	3.90	28	215	76
19	3.36	18	182	70	3.60	30	185	80
20	3.54	12	190	68	3.65	16	186	70
21	3.10	15	173	50	3.15	10	177	65
22	2.98	10	179	65	3.10	16	180	72
23	4.15	21	217	75	4.25	24	224	69
24	3.96	22	210	64	4.04	32	205	74
25	3.70	24	192	70	3.78	22	197	74
26	4.30	38	232	82	4.35	42	228	88
27	3.41	15	180	60	3.40	21	188	76
28	3.60	29	176	91	3.74	20	180	80
29	3.88	30	192	80	4.10	24	198	76
30	3.55	12	189	70	3.6z0	22	194	79
Mean ±SD	3.70 ±0.37	26.27 ±12.71<	196.93 ±19.76	73.10 ±12.99	3.72 ±0.39	25.23 ±9.51	198.73 ±17.19	71.17 ±11.12

CALUR=Carotid artery luminal diameter right side; CAIR=Thickness of carotid artery *intima* right side; CAMR=Thickness of carotid artery medial right side; CAAR=Thickness of carotid artery *adventitia* right side; CALUL= Thickness of carotid artery *intima* media left side; CAIL=Thickness of carotid artery *intima* left side; CAML=Thickness of carotid artery *media* left side; CAAL=Thickness of carotid artery *adventitia* left side.

with thickened *intima* at one site in all the sections. The *intima* exhibits numerous elastic fibrils (reduplications) of the internal elastic lamina with circularly arranged smooth muscle cells seen in the deeper part of the *intima*. The smooth muscle cells in the *tunica intima* are arranged in two layers, longitudinally cut smooth muscle cells in the deeper part of *intima*, and circularly arranged smooth muscle cells in the superficial part of the *tunica intima* (Fig. 4). At another site in the same artery the reverse pattern of arrangement of smooth muscle cells (outer longitudinal and inner circular) is seen in the *tunica intima* (Fig. 5). The *media* is composed of obliquely running smooth muscle cells arranged in two layers with few elastic fibres intervening. A special feature is the presence of numerous capillaries in these regions of thickened subendothelial tissues in the *tunica intima* (Figs. 4, 6).

Discussion

The intracranial internal carotid arteries are one of the feeder vessels supplying the brain. The intracranial and extracranial parts of these arteries show structural differences. These changes in the vessels with age in different populations can be correlated with their gross measurements and histological features.

In angiographic studies Bauer et al. (1961) observed tortuosity and kinking in 24% cases, and Oschner et al. (1977) reported coiling in three cases in the extracranial part of the internal carotid artery. These coils and kinks were seen to be sites of obstruction of blood flow. In the present study the curves which we have called tortuosities were never seen to be so sharp as to functionally obstruct the lumen. Probably the tortuosities seen by Bauer et al. (1961) were due to atherosclerosis and those seen by Oschner et al. (1977) were due to elastic tissue dysplasia. But in our study the arteries were studied in apparently healthy individuals, who had succumbed to road accidents. The tortuosities in this study may be a normal occurrence in the Indian population, because of the sinuous course of the vessel during development.

The arteries which showed these curves were longer than the arteries running straight in their course. The length of the arteries in individuals of 20-40 years of age was 21.12±2.46 mm on the right side and 21.57±2.46 mm on the left side; and in cases of more than 40 years of age 21.09±2.15 mm on the right side and 21.70±2.24 mm on the left side. In both the groups the length was found to be significantly greater on the left side. The length of the vessels in individuals of less than 20 years could not be statistically analysed, as the sample size was too small. We have found no literature where the length of this segment has been studied.

The outer diameter of the internal carotid artery in individuals of less than 20 years of age was 3.5±0.3 mm on the right side, and 3.56±0.13 mm on left side, in cases of 20-40 years of age 4.196±0.45 mm on the right side, and 4.22±0.469 mm on the left side, and in cases of more than 40 years of age 5.03±0.49 mm on the right side, and 5.12±0.57 mm on the left side. In individuals older than 40, the outer diameter on the left side was significantly more than on the right side. There is an increase in the diameter of the internal carotid arteries with age, which coincides with the diameter documented by Ratinov (1964), who recorded it as 3-5 mm. However, he did not consider the side of the artery. The increase in outer diameter could be attributed to atherosclerosis or to loss of elasticity of the wall.

The mean luminal diameter of the internal carotid artery was found to be 3.67±0.38 mm on the right side, and 3.72±0.41 mm on the left side. Blumenthal et al. (1954) have recorded average internal radius 0.066 mm (lumen = 0.132 mm with wall thickness 0.26 mm). Their study was conducted on Caucasians and African-Americans in the age group of 23-45 years, and this racial difference might be the cause of the decreased lumen diameter. The racial difference could account for the decreased diameter in their study.

In this study the intracranial carotid arteries were seen to exhibit:

1) a prominent internal elastic lamina.

2) *tunica adventitia* composed mostly of collagen fibres with few elastic fibres.

3) *tunica media*, composed of circularly arranged layer of smooth muscles with scant elastic fibres in between them.

Similarly observations were earlier reported by Triepel (1897), Fang (1958), Ratinov (1964), Pfeiffer (1972), Wilkinson (1972) and Winckler (1972).

It is well documented that in muscular arteries the endothelium rests directly on the internal elastic lamina (Gabella, 1995). In our study it is seen that subendothelial tissue is present in apparently healthy individuals and shows variable amount of thickness. Focal fibroelastic masses in excess of one fifth the thickness of the wall were seen in three cases. Similar fibroelastic masses have been documented by Blumenthal et al. (1954) in the subendothelial tissue in basilar arteries in nine of twenty-six cases. Tuthill (1930) documented these in six of the twenty cases he studied. Similar findings in the present study suggest that fibroelastic masses begin to form in younger age groups. Substantial increase in the elastic fibres in the *intima* found in patients along with migration of smooth muscle cells are distinct aging changes documented by Simionescu and Simionescu (1977).

The internal elastic lamina showed reduplication in twelve of the thirty cases studied. It splits from few to many layers. In between the layers longitudinally running smooth muscle cells and connective tissue fibres were seen.

Tuthill (1930), Hassler (1962) and Rotter et al. (1955) documented similar areas of splitting with fibroelastic masses in between the split fibres in the large cerebral arteries. However, their studies were carried out 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. Blumenthal et al. (1954) found similar sites of reduplication: the presence of multiple split connective tissue fibres and circularly arranged smooth muscle cells in between these reduplications suggest that these are early aging changes.

The external elastic lamina was observed to be an ill defined membrane made up of fragmented wavy elastic fibres. Similar reports have been made by Blumenthal et al. (1954) and Ratinov (1964). These findings thus suggest that the external elastic lamina is not a well defined layer in intracranial vessels.

The intracranial internal carotid arteries are predisposed to atherosclerotic changes and hemorrhage (Moossy, 1966; Ratinov, 1964; Blumenthal et al., 1954; McGee et al., 1962; Stein et al., 1962; Fisher et al., 1965; Ogata, 1994). Well-defined plaques have been described by Blumenthal et al. (1954), McGee et al. (1962), Stein et al. (1962), Ratinov (1964), Fisher et al. (1965), Moossy (1966) and Ogata (1994). Blumenthal et al. (1954) in 40-59 years of age observed true plaque formation consisting of focal thickening of *intima* in 30% of thickness of wall. Ogata et al. (1994) found plaque rupture, intramural hemorrhage, ulceration and embolus formation in large intracranial vessels in 8 patients in 61-77 years age group. But the patients studied were documented cases of atherosclerosis. Stein et al. (1962) and McGee et al. (1962) studied stenotic lesions of carotid vessels, but their observations were made in individuals above 50 years of age and they studied both the intracranial and extracranial parts of the vessel.

Moossy et al. (1966) found the gross lesions of atherosclerosis (fatty streaks and fibrous plaques) in intracranial internal carotid arteries in the third decade of life. More vessels were involved on the left side. The arteries of patients below 39 years of age were relatively free of thrombus formation. In the present study, variable amount of thickening in *tunica intima* with reduplication of internal elastic lamina and increased connective tissue fibres were seen in cases as young as 28 years. The vessels were found to be free of thrombus formation up to 40 years of age. More vessels were involved on the right in this study. It could be summarized that cerebral vessels are an early site of atherosclerotic involvement where the earliest changes begin from the third decade.

A special feature of the involved vessels in the present study was the presence of numerous capillaries in these areas of thickening subendothelial tissue. The presence of these vessels suggested the need for revascularization of the intima which normally receives its blood supply from the lumen. The reason why these capillaries appeared needs to be investigated.

Conclusion

The intracranial part of internal carotid arteries was longer and thicker on the left side in individuals 40-60 years of age. Thickenings of the subendothelial tissue, splittings of the internal elastic lamina, and migration of smooth muscle cells into the intima are the preatherosclerotic changes seen in healthy individuals in the third decade of life. These ageing changes in individuals in the third decade of life are an alarming sign for the Indian population.

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