Original Article

Histomorphometric and sympathetic innervation of the human superficial temporal artery

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ABSTRACT

Context: Following microvascular surgeries, stenosis and spasm of the arterial graft or the recipient vessel are serious complications which are often caused by intimal hyperplasia and perivascular nerves, respectively. **Aims:** The purpose of this study was to understand the characteristics of arterial wall and sympathetic innervation of the human superficial temporal artery (STA) and also, the effect of aging on STA. **Methods and Materials:** Fifty-two fresh human STA (frontal branch) samples were obtained from 26 cadavers (19 males and 7 females) between the ages of 19 and 83 years. Samples were divided into three age groups: G1, 19-40 years; G2, 41-60 years; G3, over 61 years. 5μm-thin sections of each sample were taken and stained with haematoxylin-eosin, Verhoff's and tyrosine hydroxylase (TH) immunostaining. **Results:** The well-defined internal elastic lamina (IEL) was observed in all samples of STA, whereas external elastic lamina (EEL) was not prominent in almost all cases or absent in few cases. This might be the important factor in the process of intimal and medial hyperplasia in the frontal branch of STA. Notably, intimal thickening appeared from second decade of life. Sympathetic fibres are located mainly in tunica adventitia and outer media. Mean adventitial and sympathetic areas were found to be 0.080 and 0.010mm^2, respectively. **Statistical analysis used:** One-way ANOVA followed by Tukey HSD *post hoc* test by using the SPSS 11.5 software. **Conclusions:** STA is prone to age related pathological changes. Sympathetic index may be used for analysis of sympathetic fibre-related problems (vasospasm, migraine) of the STA.

KEY WORDS

Aging; histology; intimal thickening; sympathetic nerves; tyrosine hydroxylase; vasospasm

INTRODUCTION

Graft selection and choice of recipient vessel are vital steps in the planning of microvascular surgeries. In recent times, the superficial temporal artery (STA) has been used as a recipient artery for the craniofacial and scalp reconstructions for several reasons, including its accessibility and its position and in caliber.[1,2] STA has also been used as a graft for STA-MCA (middle cerebral artery) bypass surgery. The first STA-MCA bypass was performed in a human by Yasargil, on a patient with a complete occlusion of the MCA.[3] STA-MCA bypass has been used for a variety of conditions that obstruct cerebral blood flow, including cerebrovascular ischaemic disease, Moyamoya disease, cerebral aneurysms,

cerebral tumors involving the proximal vasculature and other causes of diseases of the vessels.[4,5]

Sympathetic nerves of STA might be the cause of the vasospasm after STA-MCA bypass.[6] The perivascular innervation of the STA has been studied by immunohistochemistry and retrograde tracing in the rat.[7] Sympathetic nerves generally bring about vasoconstriction in most of the blood vessels, utilizing noradrenaline as a neurotransmitter. Antibodies to tyrosine hydroxylase (TH; a rate-limiting enzyme involved in the synthesis of catecholamines) are used to label the sympathetic nerves in human cadaveric cerebral arteries.[8]

To the best of our knowledge, there have been only a few studies on histomorphometry and sympathetic innervation of human STA. Stenosis and spasm of the arterial graft or recipient artery is a serious complication following microvascular reconstructive surgeries. It is often caused by intimal hyperplasia and perivascular nerves of the vessel. Hence the main purpose of this study was to investigate the characteristics of arterial wall and sympathetic innervation of the human STA.

MATERIALS AND METHODS

Sample collection
Fifty-two fresh STA (frontal branch) samples were collected from 26 cadavers during autopsy, (19 males and 7 females) between the ages of 19 and 83 years. Distribution of STA samples are shown in Table 1. This study was approved by the Kasturba hospital Ethics committee. Samples were divided into three age groups: Group 1 (G1), 19-40 years; group (G2), 41-60 years; and group 3 (G3), over 61 years. All the samples were processed for histomorphometric study and five out of 52 arteries were processed for the TH immunohistochemical staining.

Tissue processing for histological methods
All the samples were immediately fixed with 4% paraformadehyde for 24 hours and processed for histological methods without any delay. Samples were dehydrated in 50, 70, 90 and 100% alcohol, cleared in xylene, impregnated with paraffin and then embedded in paraffin. Five micrometer section were taken with rotary microtome and mounted on gelatin-coated slides and stained with haematoxylin-eosin (H and E) and Verhoff's stains.

Tissue processing for immunohistochemical method
Paraformaldehyde-fixed samples were cryoprotected in PBS containing 20% sucrose for 24 hours and then mounted with tissue-freezing medium. Five micrometer thick sections were taken by using Leitz cryostat at -20⁰C and collected onto the APES (3-Aminopropyl Triethoxysilane)-coated slides.

TH Immuno staining
Section were washed in PBS (2 x 5 minutes), treated with peroxidase block for 30 minutes, and then washed in PBS (2 x 5 minutes). Following this, the sections were blocked with normal goat serum for 1 hour, followed by incubation in rabbit polyclonal anti-TH primary antibody (AB-152,Millipore, Temecula, CA, U.S.A) diluted to 1:100 in PBS for 48 hours at 4⁰C. Sections were washed in PBS (2 x 5 minutes), incubated in biotinylated goat anti rabbit secondary antibody (sc2051, Santa Cruz, CA, U.S.A) for 2 hours followed by incubation in the HRP-streptavidin (sc2051, Santa Cruz) complex for 2 hours. Finally, colour was developed by treating these sections with DAB (sc2051, Santa Cruz) for 5 minutes. The sections were then washed with distilled water, counterstained with haematoxylin and dehydrated with two changes of alcohol, cleared in xylene and cover-slipped.

Human adrenal glands were used as positive controls and processed as above at the same time. For the negative control, sections were incubated in normal goat serum replacing primary antibody.

Morphometric analysis
Stained sections were observed under binocular light microscope and digital images were obtained. Digital images were analyzed for histomorphometric parameters [thickness of tunica intima (Ti), media and quantification of adventitial and sympathetic nerve fibre areas]. Thickness of Ti and

Table 1: Distribution of human STA samples

Age groups	No.of male cadavers	No. of female cadavers	Total no. of cadavers	No. of arteries collected	
				Right	Left
Group 1 (19-40)	6	3	9	9	9
Group 2 (41-60)	7	2	9	8	8
Group 3 (≥ 61)	6	2	8	8	8
Total	19	7	26	26	26

tunica media (Tm) were measured by using Leica Qwin V3 software. Thickness of Ti and media were measured at the five random places and then mean was obtained. Adventitial area and sympathetic nerve fibre content was obtained by in-house-developed software named "Tissue Quant" (TQ, Version 1.0), which is designed for colour quantification in Manipal Centre for Information Science, Manipal.

Statistical analysis

Statistical analysis was performed using the SPSS 11.5 software. Data were expressed as mean ± SD (standard deviation) and 95% confidence interval (CI). Data were analyzed by one-way ANOVA followed by Tukey HSD *post hoc* test. Probability (*P*) values less than 0.05 were considered significant.

RESULTS

Mean values of thickness of Ti and Tm were obtained during the histomorphometric analysis and are depicted in the Table 2. In the present study, the differences between the right and left superficial temporal arteries were not

significant; hence the mean values of right and left arteries have been taken together.

The mean thickness of Ti of G1, G2 and G3 were found to be 17.08 μ, 34.77 μ and 62.80 μ, respectively. Regarding the thickness of Ti, we found statistically significant differences in the thickness of Ti, when compared G1 with G2 ($P=\leq0.0001$), G2 with G3 ($P=\leq0.0001$) and G3 with G1 ($P=\leq0.0001$) [Table 3].

The mean, SD, 95% CI (lower bound and upper bound) and *P*-values of thickness of Tm of Group 1 (G1), Group 2 (G2) and Group 3 (G3) were shown in the Table 4. Concerning thickness of Tm, there was a statistically significant differences observed when compared G1 with G2 ($P=\leq0.0001$), G2 with G3 ($P=\leq0.0001$) and G3 with G1 ($P=\leq0.0001$).

Histological studies in this investigation revealed that STA is a muscular artery that showed numerous smooth muscle cells and very few elastic fibres in the Tm [Figure 1a]. Intimal thickening of the STA was found in all age groups studied. Notably, intimal thickening was observed in samples of

Table 2: Histomorphometric parameter values of the superficial temporal artery

Age	Sex	R_Ti	L_Ti	R_Tm	L_Tm	Mean_Ti	Mean_Tm
19	F	8.9	8.5	55.5	56.2	8.7	55.85
20	M	9.3	9.2	59.1	60	9.25	59.55
25	M	16.3	15.9	61.9	62.1	16.1	62
26	M	16.2	16.4	67	64.5	16.3	65.75
29	M	16.3	15.5	69.5	68	15.9	68.75
32	M	20.5	19.5	63.9	65	20	64.45
35	F	21	21.2	68	68.8	21.1	68.4
37	F	20.9	21.2	68.9	70	21.05	69.45
40	M	24.8	26	71.6	70.3	25.4	70.95
41	M	24.4	24	70.2	71.3	24.2	70.75
45	M	30.4	29.9	81	78.3	30.15	79.65
47	M	31.7	33.5	82.7	81.8	32.6	82.25
49	F	30	34.8	85.6	85.3	32.4	85.45
53	M	38.8	38.6	90.9	91.3	38.7	91.1
55	M	38.9	37.7	92.4	91.9	38.3	92.15
56	F	35	35.5	90.3	89.1	35.25	89.7
58	M	33.4	35.6	94.2	90.2	34.5	92.2
60	M	47	46.7	94.7	94.4	46.85	94.55
63	M	49.5	46.9	94.9	94.7	48.2	94.8
66	M	55.8	55.5	98.9	99.8	55.65	99.35
68	M	59.8	58.1	99.6	100.1	58.95	99.85
72	F	55.3	55.5	97.4	97.4	55.4	97.4
77	M	65.9	66.7	110	105.9	66.3	107.95
79	M	70.8	70.6	107	106.4	70.7	106.7
80	M	77	76.2	121.2	120.6	76.6	120.9
83	F	71	70.2	118.6	120.2	70.6	119.4

Table 3: Descriptive statistics of thickness of Ti of the superficial temporal artery

Groups	No. of cadavers	Mean (SD)	95% CI		P-value
			Lower bound	Upper bound	
Group 1 (G1)	9	17.08 (5.52)	12.84	21.33	G1-G2=≤0.0001
Group 2 (G2)	9	34.77 (6.30)	29.92	39.61	G2-G3=≤0.0001
Group 3 (G3)	8	62.80 (9.70)	54.68	70.91	G3-G1=≤0.0001
Total	26	37.27 (20.15)	29.13	45.41	

Table 4: Descriptive statistics of thickness of Tm of the superficial temporal artery

Groups	No. of cadavers	Mean (SD)	95% CI		P-value
			Lower bound	Upper bound	
Group 1 (G1)	9	65.01 (5.05)	61.13	68.89	G1-G2=≤0.0001
Group 2 (G2)	9	86.42 (7.70)	80.5	92.34	G2-G3=≤0.0001
Group 3 (G3)	8	105.79 (9.90)	97.51	114.07	G3-G1=≤0.0001
Total	26	84.97 (18.37)	77.55	92.39	

Table 5: Adventitial and sympathetic nerve fibre areas of the human superficial temporal artery

S No	Age	Sex	Side	Ada (mm^2)	Sympa (mm^2)	SI
1	32	M	R	0.072	0.011	0.153
2	49	F	R	0.101	0.010	0.098
3	55	M	R	0.085	0.013	0.148
4	56	F	L	0.080	0.009	0.108
5	63	M	L	0.064	0.008	0.120
Mean				0.080	0.010	0.125

STA during the second decade of life. In the present study, incidence of intimal thickness progressed with advancing age and found in all cases from the second decade of life [Figures 1 b and c]. Intimal thickening was diffuse in all the arteries with mild/moderate narrowing of the lumen [Figure 1c]. Thickened intima composed of lipid, fibrous tissue and foam cells [Figures 1b, d, e and f]. The incidence of intimal thickening is similar on both right and left sides. Internal elastic lamina (IEL) was fragmented usually in multiple areas, and could not be traced as a continuous structure in the perimeter of the vessel wall in samples of G2 and G3 [Figures 1d and e]. The IEL was observed in all segments of STA. In contrast, an imperfect and ill-defined external elastic lamina (EEL) was observed in most cases and only few strands of elastic fibres were found at the junction of Tm and tunica adventitia (Ta). The EEL was observed to be completely absent in six samples out of 52 samples studied [Figure 1c]. Medial calcification of the STA was found in three samples of G3 [Figure1e]. In most cases, the vessel lumen was reduced especially in G3 samples due to the intimal hyperplasia. [Figure 1f]

The results of the immunohistochemical studies revealed that TH-positive sympathetic nerve fibres were situated in the deeper parts of the Ta and outer media [Figure 2].

Mean adventitial (Ada) and sympathetic (Sympa) areas were found to be 0.080 and 0.010mm^2, respectively. Sympathetic index (SI) to STA was calculated by dividing the sympathetic fibre area by the adventitial area. SI was found to be 0.125 [Table 5]. SI may be useful to the clinicians to correlate and compare the sympathetic nerve problems (migraine and vasospasm) of the STA.

DISCUSSION

In the present study, STA showed intimal hyperplasia. Notably, intimal thickening was observed in arteries of young adults during the second decade of life. The changes observed were progressive and conformed with the findings of Diaz *et al*.[9] Histological studies of STA biopsied samples with MD were conducted and revealed that pathological changes include thickening of the Ti, degeneration and destruction of the smooth muscles in the Tm seen even in patients aged younger than 5 years.[10,11] Furthermore, intimal thickening may develop earlier in the superficial temporal arteries, which are frequently exposed to ultraviolet rays from the sun.[12] However, intimal thickening is a systemic process involving most artery types. With advancing age, an adaptive thickening of the intima can be observed within the human arterial system. Adaptive intimal thickening
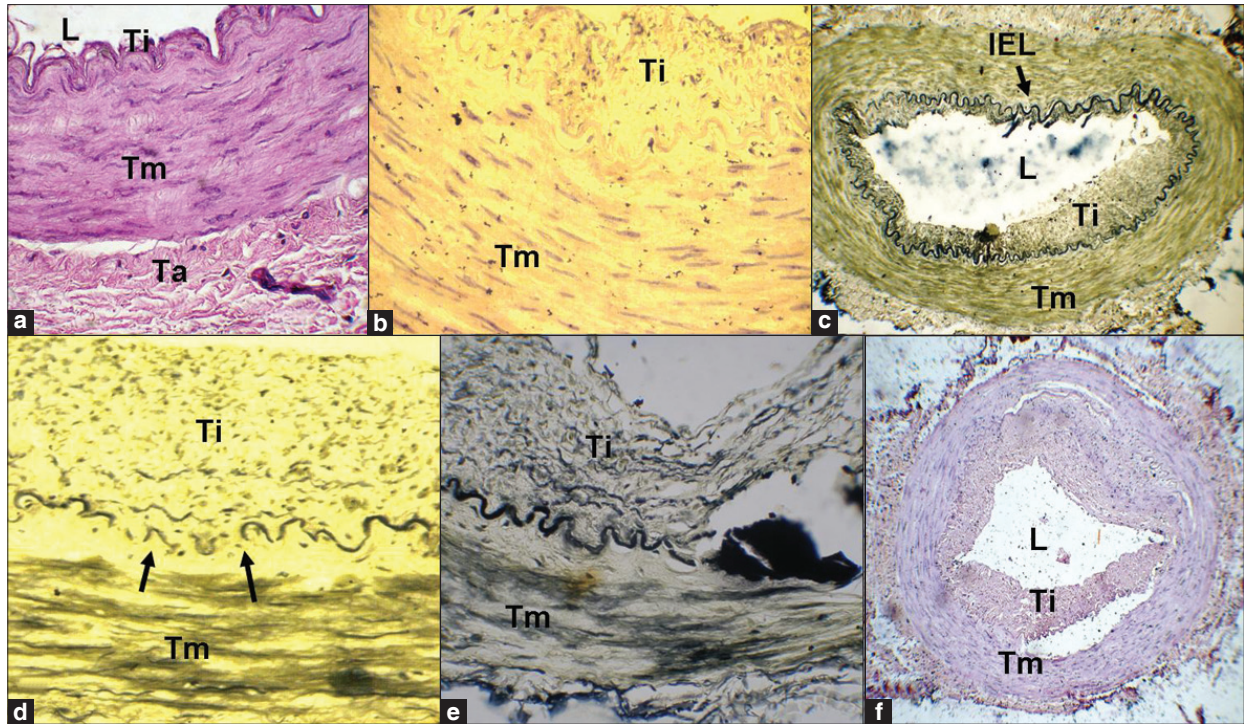
Figure 1: (a) Photomicrograph of STA of a 20-year-old individual stained with H and E, showing no intimal thickening (400X). (b) STA of 32-year-old individual stained with H and E showing intimal thickening (400X). (c) The cross section of STA of a 25-year-old individual showing IEL appears intact in the entire periphery of the vessel wall and external elastic lamina is ill-defined (100X). (d) Arrows pointing to the fragmented internal elastic lamina and thickened intima contains elastic fibres in an 83-year-old individual (400X). (e) Deposition of calcium into areas of the vessel wall which are darkly stained in a 68-year-old sample (400X). (F) STA of a 77-year-old individual stained with H and E, showing reduction of vessel lumen due to intimal thickening. There is only artifactual separation of Ti from Tm (100X), L - lumen; Ti - tunica intima; Tm - tunica media; Ta - tunica adventia; IEL – internal elastic lamina
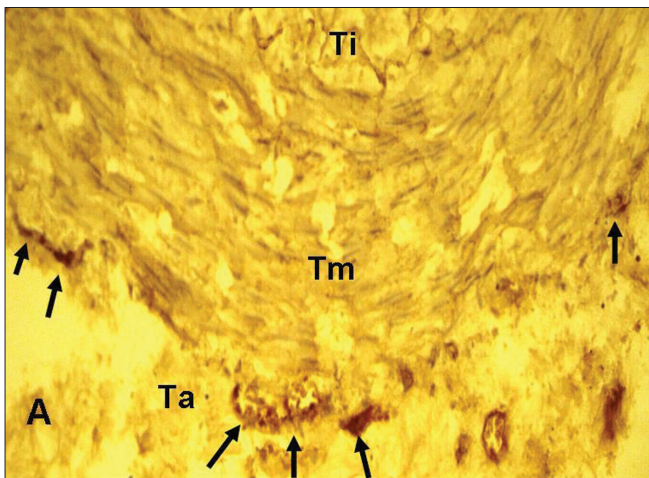

Figure 2: Arrows pointing to the sympathetic fibres in a STA of 32-year-old individual stained with TH immunostaining (400X)

could be considered as a physiological adaptation of the intima to variations in flow, wall tension or both.[13,14]

Histological studies in this investigation revealed that STA had a structure of muscular artery, showed numerous smooth muscle cells and very few elastic fibres in the Tm. Tm continues to thicken in the course of life due to the incorporation of fibrous tissue. The deposition of calcium

in the Tm was observed in samples after the sixth decade. A clinical consequence of medial calcification would be that vascular surgery becomes much more difficult. Verhoff's staining showed fragmented IEL and multilayered elastic fibres in the thickened Ti. Discontinuations in IEL were found to have increased in elderly cases. EEL is incomplete in all the cases and only strands of elastic fibres were found at the junction of Tm and Ta. In relation to the age, intimal and medial hyperplasia was observed causing the increased thickness of the arterial wall. This may be attributed to remodeling of arterial wall in response to haemodynamics and blood flow. Intimal and medial hyperplasia of the STA might be attributed to the breaks/discontinuations in the IEL. According to Sims (1985), discontinuity of the IEL causes migration of myocytes from media to intima and activates atherosclerosis.[15] However, IEL holes are necessary to provide nutrition to the Tm. Hence, if the holes are too small or if they are blocked, it may result in Tm degeneration.[16] Fragmented IEL and unclear EEL might be the cause for the increase in the thickness of Ti and Tm during the aging process. EEL serves as a barrier for cells and macromolecules between the media and adventitia in the vascular wall. A study in the coronary artery of pig suggested that atherosclerosis and vascular remodeling

may be due to the fragmentation and disorganization of EEL in porcine coronary artery.[17] Masuoka *et al.* (2010) have observed that the EEL disappeared in the cavernous part of human internal carotid artery (ICA), which is the most common site of stenosis of the intracranial ICA. Change in the elasticity of the arterial wall in the cavernous portion may be a significant reason in the process of atherosclerosis in the intracranial ICA.[18]

The facial and superior thyroid arteries were used as a recipient vessels in microvascular surgeries.[19] Histomorphometric study of the human lingual artery demonstrated an increase in thickness of intima, media, regression of IEL and luminal narrowing with increasing age.[20, 21] In the present study, age-related changes in the STA were similar to those in the lingual artery studied by previous authors.[20, 21] Shibahara and coworkers have examined histologically the recipient vessels (facial, superior thyroid, external carotid, transverse cervical and lingual artery) from the head and neck region revealed marked abnormality in intima and media in facial, external carotid and superior thyroid arteries. Hence recipient vessels should be investigated under the operating microscope with a highest magnification, prior to the anastomosis.[19]

TH immunohistochemical staining done in this investigation revealed abundant sympathetic nerve fibres in the Ta and outer media. It was observed in the rat that the STA is supplied with sympathetic nerves storing neuropeptide Y (NPY) and noradrenaline (NA), which originate from the superior cervical ganglion.[7] There are few studies available regarding the sympathetic innervation of the STA in humans. Olesen *et al.* (1995) have demonstrated in humans that STA is supplied by very rich sympathetic nerves storing NPY and NA. Vasomotor response studies indicate that the STA displayed a strong contractile response when exposed to NA, where as NPY was found to be a weaker constrictor agent. Electron microscopic studies have shown that nerve varicosities were often found in close association with the smooth muscles cells, with 100-nm-wide neuromuscular junction and fused basal lamina.[22] Results from histofluorescence and ultrastructural studies demonstrated that pial arteries are supplied by low-density sympathetic nerves, and separated widely from smooth muscle cells when compared with middle meningeal and superficial temporal arteries. By contrast, the STA has a substantial neurogenic response, because it has a high nerve density; the nerve bundles are closer to the smooth muscle cells and respond well to NA. The MMA is intermediate in its reactivity.[23] Vasospasm is seen angiographically during the first few

days following an STA-MCA bypass procedure. Allen *et al.* have studied, *in vitro*, the contractile activity of vasoactive agents on human STA and the cortical branch of MCA. They showed that the sympathetic nerves supplying STA as well as MCA may play a physiological role in determining both small and large changes in the diameter of these vessels, and postulated that the sympathetic nerves could be the cause of spasm in these arterial segments.[6] Surgical manipulation of the arteries might stimulate the sympathetic perivascular nerves causing vascular constriction and occlusion.[24] Hence perivascular nerves need to be handled gently during the surgical procedures. In the present study, sympathetic nerve fibre area was found to be 0.010 mm^2. The SI assigned to STA in this study was calculated by dividing the sympathetic fibre area by the adventitial area of STA. No data on the same parameter could be found in the available literature for comparison. SI may be used to correlate and compare sympathetic fibre-related problems (vasospasm, migraine) of STA.

CONCLUSIONS

STA is a muscular artery with ill-defined EEL. STA is supplied by sympathetic nerves which are located in the Ta and outer media. Age-related pathological changes such as intimal hyperplasia and medial calcification were observed. Hence care should be taken when considering the frontal branch of the STA as a donor artery for free microvascular flaps in the scalp and face and other head and neck regions.

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