INTRODUCTION: Internal thoracic artery (ITA) is an established arterial graft for the coronary artery by-pass surgery. Special micro-anatomical features of the ITA wall may protect it from age related pathological changes. One of the complications seen after coronary artery bypass grafting is vasospasm. Sympathetic nerves may be involved in vasospasm.

OBJECTIVE: To ascertain the sympathetic innervation of the internal thoracic artery and to assess the effect of aging on this artery by histomorphometry.

METHOD: Fifty-four human internal thoracic artery samples were collected from 27 cadavers (19 male and 8 female) with ages of 19 to 83 years. Samples were divided into three age groups: G1, 19–40 years; G2, 41–60 years; G3, ≥61 years. Sections (thickness 5 μm) of each sample were taken and stained with hematoxylin–eosin and Verhoeff–Van Gieson stains. Five of fifty-four samples were processed for tyrosine hydroxylase immunostaining.

RESULTS: The thickness of the tunica intima was found to be constant in all age groups, whereas the thickness of the tunica media decreased in proportion to age. Verhoeff–Van Gieson staining showed numerous elastic laminae in the tunica media. Significant differences (p < 0.0001) in the number of elastic laminae were found between G1 with G2 cadavers, between G2 and G3 cadavers and between G3 and G1 cadavers. Tyrosine hydroxylase immunostaining demonstrated sympathetic fibers, located mainly in the tunica adventitia and the adventitia–media border. The sympathetic nerve fiber area and sympathetic index were found to be 0.0016 mm² and 0.012, respectively.

DISCUSSION: Histology of the ITA showed features of the elastic artery. This may be associated with lower incidence of Atherosclerosis or intimal hyperplasia in ITA samples even in elderly cases. Low sympathetic index (0.012) of ITA may be associated with fewer incidences of sympathetic nervous systems problems (vasospasm) of the ITA.

CONCLUSION: Sympathetic nerve fibers are present in the adventitia of the internal thoracic artery. This is an elastic artery, although anatomically it is considered to be medium-sized. The sympathetic index may be used for analysis of sympathetic nerve fiber-related problems of the internal thoracic artery.

KEYWORDS: Histomorphometry; Aging; Sympathetic nerves; Elastic fibers; Sympathetic index.
enzyme involved in the synthesis of catecholamines) were used to label the sympathetic nerves in human cadaveric cerebral arteries. Sympathetic innervation of the ITA has been discussed in the literature and the results are controversial. Barry et al., using S-100 immunostaining, reported that sympathetic fibers are not found in the ITA. However, Deja et al., using the same S-100 immunostaining, found that sympathetic nerves were present in the ITA. Hence the main purpose of this study was:

- to study sympathetic innervation;
- to study the detailed microanatomy and histomorphometry of the ITA and the influence of age on the internal thoracic arterial wall.

MATERIALS AND METHODS

Sample Collection

Fifty-four fresh human ITA samples were collected during autopsy from 27 cadavers (19 male and 8 female) with ages of 19 to 83 years. The time elapsed between the deaths and sampling ranged between 10 and 15 hours. This study was approved by the Kasturba hospital ethics committee (KHEC/63/2006). All arterial samples were divided into 3 groups, according to age—group I: samples of those aged 19-40; group II: samples of those aged 41-60; group III: samples of those aged ≥ 61. The distribution of ITA samples is shown in Table 1. All the samples were processed for a histomorphometric study and 5 out of 54 arteries were processed for the TH immunostaining.

Method of Collection

A midline incision was made through the skin, extending from symphysis menti to the upper part of the anterior abdominal wall and the sternal and clavicular origins of the pectoralis major were detached and reflected laterally to expose the costal cartilages and ribs. The right and left clavicles were then dislocated at the sternoclavicular joints and the ribs were detached from the costal cartilages. The anterior portion of the thoracic cage (sternum with costal cartilages) was raised and the ITA was identified close to the anterior portion of the thoracic cage (sternum with costal cartilages) was raised and the ITA was identified close to the sternum. The ITA was harvested between the first and second costal cartilages.

Fixation

All the samples were washed in phosphate-buffered saline (PBS) until the lumen was free of blood clots, immediately fixed with 4% paraformaldehyde for 24 hours and subsequently, processed for histological methods without any delay.

Tissue Processing for Histological Methods

Samples were dehydrated in 50%, 70%, 90% and absolute alcohol, cleared in xylene, impregnated with paraffin and then embedded in paraffin. Sections (5 μm) were taken with a rotary microtome and mounted on gelatin-coated slides and stained with hematoxylin-eosin and Verhoeff–Van Gieson stains.

Hematoxylin and Eosin (H&E) Staining

Deparaffinized sections were rinsed with distilled water after rehydration in graded alcohol. These sections were stained with hematoxylin for 3 minutes and eosin for 1 minute. Then sections were dehydrated in two changes of absolute alcohol, cleared with xylene and cover slipped with DPX (mixture of distyrene, a plasticizer and xylene) mounting medium.

Verhoeff–Van Gieson Staining

Deparaffinized sections were rinsed with distilled water after rehydrating in graded alcohol. These sections were stained with Verhoeff solution for 10 minutes and then rinsed in running tap water. The sections were differentiated in 2% ferric chloride for 30 seconds and observed microscopically for black elastic fibers, followed by counterstaining with Van Gieson solution. Finally, the sections were dehydrated through two changes of absolute alcohol, cleared with xylene and cover slipped with DPX mounting medium.

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. Sections (5 μm) were taken using a Leitz cryostat at −20°C and collected onto the 3-aminopropyl triethoxysilane (APES) coated slides.

Tyrosine Hydroxylase (TH) Immunostaining

Section were washed in PBS (2 × 5 minutes), treated with peroxidase block for 30 minutes, and then washed in PBS (2 × 5 minutes). After this, the sections were blocked with normal goat serum for 1 hour, followed by incubation in rabbit polyclonal anti-TH primary antibody (AB-152, Temecula, CA, U.S.A) diluted 1100 in PBS for 48 hours at 4°C. Sections were washed in PBS (2 × 5 minutes), incubated in biotinylated goat anti-rabbit secondary antibody (Sc-2051, Santa Cruz, CA, U.S.A) for 2 hours followed by incubation in the horseradish peroxidase-streptavidin (Sc-2051, Santa Cruz, CA, U.S.A) complex for 2 hours. Finally, color was developed by treating these sections with 3, 3-diaminobenzidine (DAB; Sc-2051, Santa Cruz, CA, U.S.A) for 5 minutes. The sections were then washed with distilled.

Table 1 - Distribution of human internal thoracic artery samples.

| SI No. | Age groups | No. of male cadavers | No. of female cadavers | Total No. of cadavers | No. of arteries collected |
|--------|------------|----------------------|------------------------|----------------------|-------------------------|
| 1      | Group 1 (19-40) | 7                    | 3                      | 10                   | 10                      |
| 2      | Group 2 (41-60) | 5                    | 3                      | 8                    | 8                       |
| 3      | Group 3 (≥ 61)  | 7                    | 2                      | 9                    | 9                       |
|        | Total       | 19                   | 8                      | 27                   | 27                      |

SI = sympathetic index.
water, counterstained with hematoxylin, 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 in place of primary antibody.

**Analyzed Parameters**

Stained sections were observed under binocular light microscope and digital images were obtained. Digital images were analyzed for the following histomorphometric parameters:

1. The thickness of the tunica intima (Ti) and tunica media (Tm) was measured in five random places using the Leica Qwin V3 program at a magnification of ×400; a mean value was obtained.
2. The number of elastic laminae (Nel) was obtained at a magnification of ×400.
3. The adventitial area and sympathetic fiber content were obtained at a magnification of ×40 using the software developed in-house—“TissueQuant”—which is designed for color quantification. This software enables the choice of a color for selectively identifying pixels in the image with the chosen color and its shades.

For the purpose of calibration, images of a scale both in horizontal and vertical positions were obtained under the same magnification. The number of pixels representing a length of 1 mm was calculated for both horizontal and vertical arrangements. This provided the calibration for the number of pixels representing an area of 1 mm².

**Statistical Analysis**

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

**RESULTS**

In this study, the differences between the right and left arteries were not significant, hence the mean values of right and left were taken together. The mean values of thickness of Ti, Tm and Nel were obtained during the histomorphometric analysis and are shown in Table 2.

The mean, SD, 95% CI (lower bound and upper bound) and p values for the thickness of Ti in groups 1 (G1), 2 (G2) and 3 (G3) are depicted in Table 3. The differences in the thickness of Ti between G1 and G2 (p = 0.851), between G2 and G3 (p = 0.126) and between G3 and G1 (p = 0.270) were not statistically significant.

The mean, SD, 95% CI (lower bound and upper bound) and p values for the thickness of Tm in groups 1 (G1), 2 (G2) and 3 (G3) are presented in Table 4. The difference in the thickness of Tm between G1 and G2 (p = 0.883) was not statistically significant. However, a significant difference was seen between G2 and G3 (p = 0.003) and between G3 and G1 (p = 0.005).

The mean, SD, 95% CI (lower bound and upper bound) and p values of Nel in groups 1 (G1), 2 (G2) and 3 (G3) are shown in Table 5. Significant differences were seen between G1 and G2 (p≤0.0001), between G2 and G3 (p≤0.0001) and between G3 and G1 (p≤0.0001).

**Table 2 - Internal thoracic artery histomorphometric parameter values.**

| Age | Sex | R_ti (μm) | L_ti (μm) | R_tm (μm) | L_tm (μm) | R_nel | L_nel | Mean_ti (μm) | Mean_tm (μm) | Mean_nel |
|-----|-----|-----------|-----------|-----------|-----------|-------|-------|-------------|-------------|----------|
| 19  | F   | 9.16      | 8.08      | 85.58     | 91.56     | 9     | 9     | 8.62        | 88.57       | 9        |
| 20  | M   | 6.1       | 113.5     | 114.84    | 10        | 10    | 6.1   | 114.17      | 10          | 10       |
| 25  | M   | 8.54      | 8.78      | 90.15     | 90.96     | 9     | 10    | 8.66        | 90.56       | 9.5      |
| 26  | M   | 8.77      | 8.14      | 98.69     | 99.48     | 9     | 9     | 8.46        | 99.09       | 9        |
| 29  | M   | 7.74      | 8.04      | 109       | 110.42    | 10    | 9     | 7.89        | 109.71      | 9.5      |
| 32  | F   | 8.7       | 8.8       | 100.2     | 101.1     | 9     | 9     | 8.75        | 100.65      | 9        |
| 35  | F   | 8.9       | 8.5       | 107       | 107.9     | 9     | 9     | 8.7         | 107.45      | 9        |
| 37  | M   | 9.7       | 10.3      | 104.8     | 104.4     | 9     | 9     | 10.46       | 110.69      | 9        |
| 40  | M   | 9.04      | 9.28      | 122.44    | 121.58    | 9     | 8     | 9.16        | 122.01      | 8.5      |
| 41  | M   | 8        | 8.5       | 104.3     | 113.4     | 7     | 8     | 8.25        | 108.85      | 7.5      |
| 43  | F   | 6.8       | 7.3       | 111.24    | 111.5     | 8     | 8     | 7.05        | 111.37      | 8        |
| 47  | M   | 9.56      | 9         | 108.6     | 107.9     | 7     | 8     | 9.28        | 108.25      | 7.5      |
| 49  | F   | 7.5       | 7.82      | 101.24    | 100.88    | 7     | 7     | 7.66        | 101.06      | 7        |
| 53  | M   | 8.2       | 8         | 110       | 112.1     | 6     | 7     | 8.1         | 110.05      | 6.5      |
| 55  | M   | 10.18     | 10.12     | 110.12    | 106.76    | 7     | 7     | 10.15       | 108.44      | 7        |
| 56  | F   | 8.5       | 8.19      | 111.1     | 100.6     | 6     | 8     | 8.34        | 105.85      | 7        |
| 60  | M   | 7        | 7.64      | 100.8     | 98.1      | 7     | 7     | 7.32        | 99.45       | 7        |
| 63  | M   | 9.3       | 9.22      | 91.56     | 91.38     | 6     | 6     | 9.26        | 91.57       | 6        |
| 64  | M   | 8.5       | 8.6       | 103.6     | 103.5     | 5     | 5     | 8.55        | 103.55      | 5        |
| 66  | M   | 8.6       | 8.6       | 98        | 96.8      | 6     | 6     | 8.6         | 97.4        | 6        |
| 68  | M   | 10.3      | 10.7      | 94.4      | 88.6      | 6     | 6     | 10.5        | 91.5        | 6        |
| 72  | F   | 8.9       | 9.2       | 95.2      | 95.22     | 4     | 5     | 9.05        | 95.21       | 4.5      |
| 77  | M   | 8.2       | 8.36      | 90.4      | 94.98     | 4     | 5     | 8.28        | 92.69       | 4.5      |
| 79  | M   | 10.8      | 11.34     | 88.8      | 89.38     | 4     | 4     | 11.07       | 89.09       | 4        |
| 80  | M   | 9.3       | 9.1       | 88.6      | 89        | 3     | 4     | 9.2         | 88.8        | 3.5      |
| 83  | F   | 8.7       | 8.72      | 71.9      | 71.76     | 3     | 3     | 8.71        | 71.83       | 3        |

L_nel = number of elastic laminae on left side; L_ti = thickness of tunica intima on left side; L_tm = thickness of tunica media on left side; R_nel = number of elastic laminae on right side; R_ti = thickness of tunica intima on right side; R_tm = thickness of tunica media on right side.
The findings of this study showed that the thickness of Ti is constant in all age groups, whereas the thickness of Tm decreases as age advances. A histological study showed that age-related pathological changes such as intimal thickening or atherosclerosis and medial calcification are not observed in any of the ITA samples studied (Figure 1A). Tm showed few smooth muscles and numerous elastic laminae, which are arranged concentrically as in elastic arteries (Figure 1B).

Table 3 - Differences* in the thickness of Ti between the groups studied.

| Groups     | Sample size (N) | Mean (SD) (μm) | Lower bound | Upper bound | p Value  |
|------------|----------------|----------------|-------------|-------------|----------|
| Group 1 (G1) | 10             | 8.52 (1.00)    | 7.8         | 9.24        | G1–G2 = 0.851 |
| Group 2 (G2) | 8              | 8.26 (1.02)    | 7.41        | 9.12        | G2–G3 = 0.126 |
| Group 3 (G3) | 9              | 9.24 (0.93)    | 8.52        | 9.96        | G3–G1 = 0.270 |
| Total      | 27             | 8.68 (1.03)    | 8.27        | 9.10        |          |

*Differences (one-way analysis of variance followed by Tukey HSD post hoc test) were not statistically significant.

Ti = tunica intima.

Table 4 - Differences* in the thickness of Tm between the groups studied.

| Groups     | Sample size (N) | Mean (SD) (μm) | Lower bound | Upper bound | p Value  |
|------------|----------------|----------------|-------------|-------------|----------|
| Group 1 (G1) | 10             | 104.87 (10.45) | 97.4        | 112.35      | G1–G2 = 0.883 |
| Group 2 (G2) | 8              | 106.79 (4.40)  | 103.1       | 110.47      | G2–G3 = 0.003 |
| Group 3 (G3) | 9              | 91.29 (8.62)   | 84.66       | 97.92       | G3–G1 = 0.005 |
| Total      | 27             | 100.91 (10.70) | 96.68       | 105.15      |          |

The difference between G1 and G2 (p = 0.883) was not statistically significant. Note that there was a significant difference in the thickness of Tm between G2 and G3 (p = 0.003) and between G3 and G1 (p = 0.005) (one-way analysis of variance followed by Tukey HSD post hoc test). The thickness of Tm was found to have decreased in the aging process, possibly owing to a decrease in the number of elastic laminae.

Tm = tunica media.

Table 5 - Decrease in number of elastic laminae (Nel) with increasing age.

| Groups     | Sample size (N) | Mean (SD) | Lower bound | Upper bound | p Value  |
|------------|----------------|-----------|-------------|-------------|----------|
| Group 1 (G1) | 10             | 9.15 (0.41) | 8.85        | 9.44        | G1–G2 = 0.0001 |
| Group 2 (G2) | 8              | 7.18 (0.45) | 6.80        | 7.57        | G2–G3 = 0.0001 |
| Group 3 (G3) | 9              | 4.72 (1.12) | 3.86        | 5.58        | G3–G1 = 0.0001 |
| Total      | 27             | 7.09 (2.01) | 6.29        | 7.89        |          |

There were statistically significant differences in Nel between G1 and G2 (p = 0.0001), between G2 and G3 (p = 0.0001) and between G3 and G1 (p = 0.0001) (one-way analysis of variance followed by Tukey HSD post hoc test).

The findings of this study showed that the thickness of Ti is constant in all age groups, whereas the thickness of Tm decreases as age advances. A histological study showed that age-related pathological changes such as intimal thickening or atherosclerosis and medial calcification are not observed in any of the ITA samples studied (Figure 1A). Tm showed few smooth muscles and numerous elastic laminae, which are arranged concentrically as in elastic arteries (Figure 1B).

Figure 1 - (A) Photomicrograph of the internal thoracic artery (ITA) wall of a 79-year-old subject stained with hematoxylin and eosin showing no age-related pathological changes (×400). (B) ITA of a 20-year-old subject stained with Verhoeff–Van Gieson stain, showing numerous elastic laminae in the Tm (×400). (C) ITA of an 83-year-old subject showing decreased number of elastic laminae in the Tm and discontinuous IEL (×400). IEL = internal elastic lamina; L = lumen; Ti = tunica intima; Tm = tunica media.
This study also showed a decrease in the number of elastic laminae during the aging process. The internal elastic lamina (IEL) was continuous in all young samples of the ITA, while discontinuations in the IEL were seen in the samples above 60 years (Figure 1C).

TH immunostaining showed that sympathetic nerve fibers are present in the tunica adventitia and at the adventitia–media border (Figure 2A and B). The adventitial and sympathetic nerve fiber areas of the ITA are shown in Table 6. The mean adventitial and sympathetic nerve areas were 0.134 mm$^2$ and 0.0016 mm$^2$, respectively. The sympathetic index (SI) for the ITA was calculated by dividing the sympathetic fiber area by the adventitial area. The mean SI value was found to be 0.012. SI may be used for the sympathetic fiber-related problems of the ITA.

DISCUSSION

In this study, Ti was well developed in all cases. The mean thickness of Ti in age groups 1, 2 and 3 were 8.52 μm, 8.26 μm and 9.24 μm, respectively. The thickness of the Ti appeared to be constant during the aging process. The IEL represent a flexible barrier between the endothelium of Ti and the inner smooth muscle cell layer of Tm and may have a role in atherogenesis via its modulation of diffusion across the artery wall. According to Sims, discontinuity of the IEL causes migration of myocytes from media to intima and activates atherosclerosis. However, internal elastic lamina holes are necessary to provide nutrition to the Tm. Hence, if the holes are too small or if they are blocked, that may result in Tm degeneration. The results of this study show that intimal thickening or atherosclerosis was not seen in the ITA, though discontinuations are seen in ITA samples above 60 years of age. This may be because the ITA has natural immunity to atherosclerosis, active vasodilatation is accompanied by remodeling of the arterial wall, it has a great capacity for nitric oxide secretion and other endothelial factors, regulating the vasomotor tonus and the auto-repair mechanism. Reports of Sandow et al. have suggested that internal elastic lamina hole density is not related to hypertension or intimal lesion formation in rats.

The Tm of the ITA showed few smooth muscle cells and numerous elastic laminae, which are arranged concentrically like elastic arteries. The mean number of elastic laminae (including internal and external elastic laminae) in groups 1, 2, and 3 were 9.15, 7.18, and 4.72, respectively. The thickness of the Tm was found to have decreased during the aging process, possibly owing to a decrease in the number of elastic laminae during aging. Standard text books of histology classify arteries based on their diameter, into arterioles, medium or muscular arteries and larger or elastic arteries. The Tm of a large artery contains chiefly elastic fibers and few smooth muscles, whereas the Tm of medium-sized arteries contains predominantly smooth muscle cells and fewer elastic fibers. In this study, Verhoeff stain revealed that the ITA is an elastic artery, though anatomically, it is considered to be medium-sized. Similar results have been reported by others. The ITA is considered as an elastic artery in the proximal and middle part, whereas only the distal part is considered as muscular artery. However, Marx et al. examined the distal part of the ITA in 100 patients and found an elastic type of vessel, but hybrid and muscular patterns of the ITA have also been reported in the literature. In this study, the ITA was dissected between the first and second costal cartilage, which can be considered as being in between the proximal and middle part of the ITA.

TH immunohistochemical studies revealed that sympathetic fibers are situated mainly in the tunica adventitia and at the adventitia–media border. The mean sympathetic nerve area of the ITA was found to be 0.0016 mm$^2$. The findings of this study are in contrast with those of Barry et al. who used S-100 immunostaining and reported that sympathetic fibers are not present in the ITA. However, Gaudino et al. using TH immunostaining, and Deja et al. using S-100 immunostaining, did find sympathetic fibers in the adventitia of the ITA.

In our study, no direct intimate contact between the smooth muscles of the Tm and sympathetic fibers was observed. This may have resulted in a lower incidence of

### Table 6 - Adventitial and sympathetic nerve fiber area of the internal thoracic artery (ITA).

| Sl. No. | Age (years) | Sex | Side | Ada (mm$^2$) | Sympa (mm$^2$) | Sl |
|---------|-------------|-----|------|-------------|-------------|----|
| 1       | 40          | M   | Right | 0.125       | 0.002       | 0.016 |
| 2       | 43          | F   | Left  | 0.115       | 0.001       | 0.009 |
| 3       | 56          | F   | Right | 0.149       | 0.002       | 0.013 |
| 4       | 77          | M   | Right | 0.133       | 0.001       | 0.008 |
| 5       | 80          | M   | Left  | 0.148       | 0.002       | 0.014 |
| Mean    | 59.2        | M   | Right | 0.134       | 0.0016      | 0.012 |
| SD      | 18.65       | M   | Right | 0.014       | 0.0005      | 0.003 |

The sympathetic index (SI) for the ITA was calculated by dividing the sympathetic fiber area by the adventitial area. 
Ada = adventitial area; Sympa = sympathetic area.
vasospasm in ITA grafts. Barry et al.10 reported sympathetic and parasympathetic nerve fibers in the radial, ulnar, epigastric and coronary arteries. These sympathetic and parasympathetic nerve fibers may be responsible for vasospasm of coronary, radial, ulnar and epigastric arteries. According to Suma and Fisk et al., the tendency for vasospasm is higher in the gastroepiploic and radial arteries than in the ITA.25,26 The incidence of spasm during harvesting may also be related to technique. Gentle manipulation may reduce the incidence of spasm, but there is no evidence to show that spasm can be totally avoided by gentle harvesting without pharmacologic intervention.27 Knowledge of the way in which vasospasm develops is still lacking. However, it is presumed that vasospasm is an extreme form of vasoconstriction, which may be the response of a vessel to many stimuli, which may be physical (mechanical stimulation or temperature changes) or pharmacologic (nerve stimulation or vasoconstrictor substances).27

The SI assigned to the ITA in this study was calculated by dividing the sympathetic fiber area by the adventitial area of the ITA. No data on the same parameter could be found in the literature for comparison. SI may be used to correlate and compare sympathetic fiber-related problems (vasospasm) of the ITA following coronary artery bypass surgery.

CONCLUSION

It can be concluded that the ITA is an elastic artery though anatomically it is considered to be medium-sized. Sympathetic fibers exist in the adventitia of the ITA. The thickness of the Ti appeared to be constant, where as the thickness of the Tm and number of elastic laminae decreased during the aging process. Age-related pathological changes like atherosclerosis, intimal hyperplasia and medial calcification are not seen in any of the ITA samples. The SI may be used for the sympathetic fiber-related problems of the ITA.

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