Quantitative study of elastin in human arteries with a high level of tortuosness

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Summary

The present study analysed 63 human arteries of similar tortuousness taken from uterine, splenic, superficial temporal, lingual and facial arteries. We considered morphometric aspects related to intimal disease (Intimal Thickening Index and Elastolyse Index) and elastin concentration in the medial and intimal layers and thickenings. Although their elastin concentrations were similar, different groups were detected when we compared the indices. We postulate the histological organisation presented and the involvement of other elements such as the hemodynamical variations in blood flow and the production of matrix extracellular substances are significant elements which will be assessed in future investigations.

Key Words: Arterial morphometry - elastin - intimal thickenings - quantitation color-images.

Introduction

Elastin, one of the constituents of the extracellular arterial matrix, is the essential component of elastic fibbers. It is formed during the second half of gestation, after birth and during the early neonatal period. After this stage of active synthesis, its production rapidly decreases under normal conditions, so that elastin seems to remain constant there after. Recent feature increase the role played by the elastin organisation and their distribution through the arterial wall in differents territories of the arterial tree (Li et al., 1998; Avolio et al., 1998; Lillie et al., 1998).

The collagen/elastin ratio increases as the artery ages (Dobrin et al., 1994). In some arterial diseases such as atherosclerosis, alterations in the organisation of elastin are detected (Ortiz et al., 1998). In differents human arteries territories, the changes compared with atherosclerose are not the same in all cases. Some arteries withstand the aging process and consequent intimal thickening better than others. Arteries which present the greatest tortuousness tend to be the most severely affected (Robert et al., 1986).

We selected a group of human arteries with a high level of tortuousness (Adair et al., 1994) to study the elastic component of their medial layer and compared them with the presence or absence of intimal disease. We analysed the amount of elastin and morphological peculiarities of each area to explain the differences found in comparison with intimal disease and associated the changes with other factors which may prove useful in subsequent studies.

Materials and Methods

In all, 63 human arteries were tested, taken from medical legal autopsies of subjects ranging in age from 40 to 60 years. After background clinical investigation, they had not died of degenerative or invasive vascular processes, lipid change or diagnosed high blood pressure.

The territories studied and the samples taken from each of these subjects involved the following arteries: uterine (n=17), splenic (n=10), superficial temporal (n=14), lingual (n=10) and

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facial (n=12). This arteries presented the same level of tortuosity according the classification of Adair et al. (1994). After dissection, each piece was sectioned transversally in 2 cm-long segments. Only the medial and intimal layers were considered. The pieces were fixed following customary protocol for each case, cut using Leitz microtome at 5-7 μm. and stained using Orceine and Verhoëff technique for elastic fibbers.

For video analysis the sections are visualised by the operator through either an upright or an inverted Nikon microscope and capture through a colour CCD camera; the video signal is digitised with a Matrox "Meteore" device and saved onto a computer disk. In order to have reproducibility between image capture, we controlled the light intensity through the tissue specimen; each image was calibrated by its histogram values of the three color (Red, Green, Blue). The morphometric study and colour intensity staining was carried out in INSERM Unit 441, Pessac-France using the QUANCOUL program (Daniel Lamazière et al., 1993) at power magnification of X400. The software Quancoul (Quant'Image-INSERM U441, France) defined true colours on the basis of three independent parameters (Hue, Intensity, Saturation). The parameters were calibrated against a background of control histological technique. The thresholds were adjusted so that all but the faintest staining could be detected. Since the detected color amount is expressed by both the number of the stained pixel (% of the labelled surface) and the intensity of coloured.

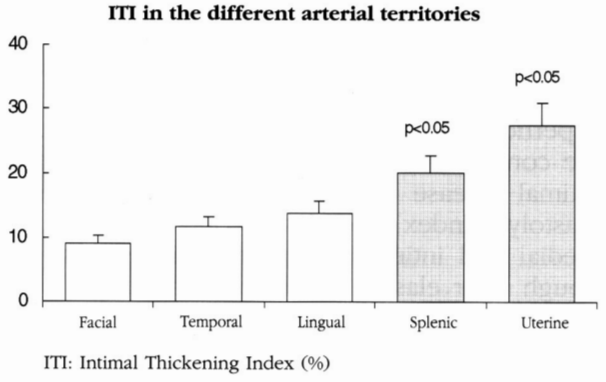
Each case was analysed on three different slides, each slide contains five different sections. The medial and intimal layers were measured for morphometric evaluation and the elastin for the quantitation in these areas.

Statistical study, we analysed the mean, standard deviation, variance and Newman Keuls q for $p<0.05$ and $p<0.01$

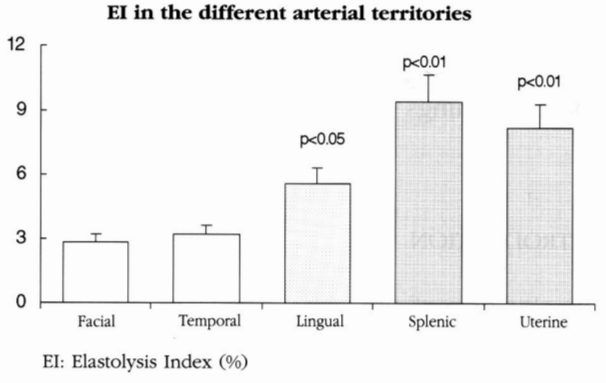
We defined the Intimal Thickness Index (ITI) as the ratio of the intimal surface divided by medial surface; Elastolysis, the mean distance between two Internal Elastic Laminae (IEL) fragments; the Contour or mean lineal distance of the IEL for a single surface studied and the Elastolysis Index (EI) was defined as the ratio between the values of elastolysis and the mean contour of the IEL. The ITI can be proposed as an index of arterial thickening and the EI as an index of elastin organisation.

Results

The morphometrical measurements obtained are showed in table 1. In relation to morphometric parameters, the statistical analysis confirm the existence of significant differences. In the ITI (Graph 1) we found 2 extensive arterial groups, one made up of the superficial temporal, lingual and facial arteries (cranial region) and the second, uterine and splenic arteries (abdominal region). The arteries of the cranial territory presented significantly lower values that the abdominal territory, with higher values ($p<0.05$). The study of the integrity of the IEL, through the EI (Graph 2) put the arteries studied in the same two groups, cranial and abdominal territories. In the cranial group, the temporal and facial arteries presented the lowest values but the EI in the lingual artery are significantly more elevated ($p<0.05$). The highest values of EI are observed in the abdominal arteries group ($p<0.01$).



Graph 1.



Graph 2.

In relation to elastin quantitation, the corresponding values to the concentration of elastin among the different layers (medial and intimal, respectively) did not present significant differences into two groups.

Elastin distribution in the arterial wall presented differences and particularities into two groups. In the abdominal group (uterine and

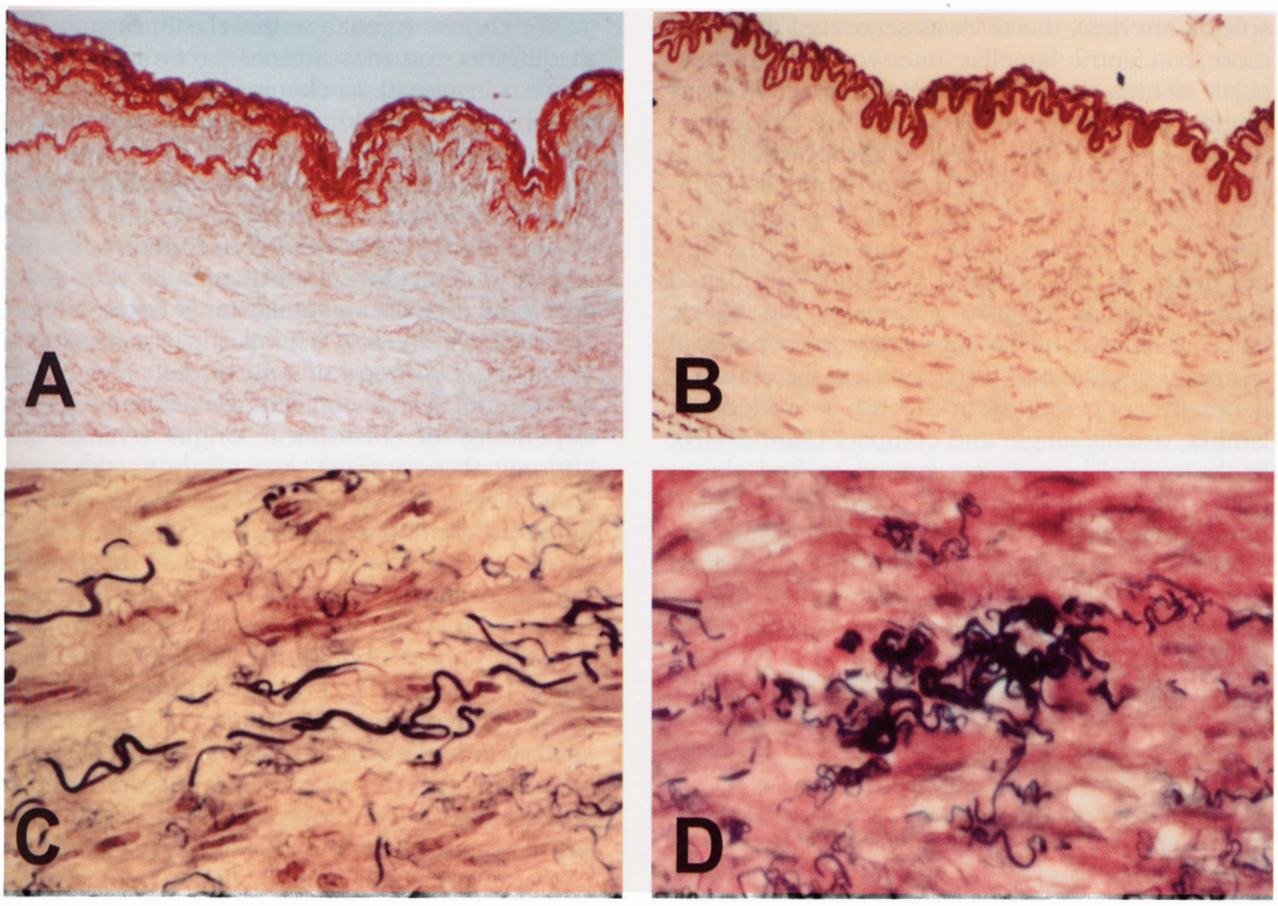


Fig. 1.– A) Uterine artery (x20). IEL of the arteries with high ITI and EI: frayed in concentric lamellae with different thickness and number. Orceine technique.
B) Lingual artery (x10). I.E.M. of the arteries with less ITI and EI: vigorous, single and compact. Orceine technique.
C) Elastic fibers in the medial layer (x40): isolated and finely undulated, corresponding arteries with high ITI (abdominal group). Verhoëff technique.
D) Facial artery: Polymorphism of elastic fibbers in the medial layer (x40): irregular undulations, rolled and elastic knots, corresponding arteries with less ITI (cranial territory). Verhoëff technique.

Table 1.

Summary of morphometric data in the different arteries studied

	Abdominal arteries		Cranial arteries		
	Uterine	**Splenic**	**Temporal**	**Lingual**	**Facial**
Media	1,29 ± 0,4	2,81 ± 0,8	0,62 ± 0,2	0,74 ± 0,2	0,87 ± 0,5
Intima	0,28 ± 0,03	0,11 ± 0,04	0,02 ± 0,01	0,04 ± 0,0	0,01 ± 0,0
Thickening	0,38 ± 0,1	0,26 ± 0,3	0,1 ± 0,03	0,06 ± 0,0	0,26 ± 0,07
ITI	27,38 ± 5,8*	20,02 ± 4,9*	11,71 ± 3,3	14,86 ± 3,1	7,14 ± 2,8
Contour	316 ± 15	404 ± 82	250 ± 18	267 ± 24	250 ± 12
Elastolysis	26 ± 6,3	38 ± 8,9	8,5 ± 3,2	15 ± 5,6	6,9 ± 2,5
EI	8,21 ± 1,0**	9,45 ± 2,9**	3,2 ± 1,2	5,6 ± 1,9*	2,8 ± 0,1
E. Media	16,4 ± 4,3	13,73 ± 4,0	11,22 ± 4,2	16,8 ± 3,9	13,1 ± 3,3
E. Intima	80,8 ± 3,6	76,4 ± 6,8	79,76 ± 8,1	72,6 ± 7,5	79,6 ± 3,5
E. Thickening	50,6 ± 8,7	41,3 ± 10	51,48 ± 11	48,3 ± 10	60,2 ± 4,3

Media: Surface of the medial layer in mm^2. **Intima:** Surface of the intimal layer in mm^2. **Thickening:** Surface of the arterial thickening in mm^2. **ITI** (Intimal Thickening Index): Ratio of the intimal surface divided by medial surface expressed in %. **Contour:** Mean lineal distance of the IEL for a single surface in µm. **Elastolysis:** Mean distance between two Internal Elastic Laminae (IEL) fragments expressed in µm. **EI** (Elastolysis Index): Rato between the values of elastolysis and the mean contour of the IEL, expressed in %. **E. Media:** Quantification color of elastin in the medial layer expressed in % surface occuped. **E. Intima:** Quantification color of elastin in the intimal layer expressed in % surface occuped. **E. Thickening:** Quantification color of elastin in the arterial thickening (%).
p values: * $p<0.05$, ** $p<0.01$

splenic arteries), the IEL was separated in two or more concentric lamellae interwoven with muscular strands, in differents laminae thickenings (Figure 1-A). In the areas underlying the intimal thickenings, the IEL integrity disappeared and was substituted by a fragmented and disorganizated elastin. In the medial tunic, the elastic fibbers were distributed more frequently in the outher media as thin, isolated and finely undulated elastin lamellae (Figure 1-C) near the elastic external lamina. In the cranial group (superficial temporal, lingual and facial arteries) the elastin is generally concentrated in the IEL as a vigorous lamina, usually single and compact (Figure 1-B). In the medial layer it is common to find elastin fibbers in the inner media, as small undulated lines among the SMC and following a concentric disposition. A great deal of polyforms is added to this distribution, randomly distributed fibbers with irregular undulations forming elastic knots (Figure 1-D), a tree aspect or even very fine perpendicular lines in a radial direction from the IEL.

Discussion

The production of elastin, an essential component of the extracellular matrix, occurs mainly in the foetal period and first stages of development (Pierce et al., 1995). Extracellular matrix plays an important role to maintain the normal properties of the arterial tree (Daniel Lamazière et al., 1997). Foetal arterial wall present the gene of tropoelastin expression in vascular smooth muscle cells (SMC) (Stenmark et al., 1994). After the fifth month of the gestation, the accumulation of elastin is accelerated in all the arteries but most rapidly in the thoracic aorta (Bendeck et al., 1994). During natal and perinatal development, the role played by elastin is crucial to control vascular integrity (Curran et al., 1993; Li et al., 1998). In the muscular arteries, the elastic material is concentrated in the internal and external elastic laminae. Less elastin is found in the medial layer.

With age, the extracellular matrix changes its concentrations of collagen and elastin, thus causing fragmentation of the elastic fibbers and arterial rigidity (Ooyama et al., 1995). In atherosclerose, elastin loses also its properties by specific enzyme degradation such as elastase, proteins secreted by the dedifferentiated SMC (Patel et al., 1996). These elastase attack the IEL elastin and permits the migration to the SMC from the neointimal space. In the medial layer, the elastin disorganisation increased the dilatation and elongation, producing an increase of arterial tortuosity (Dobrin et al., 1994) and more aneurysms incidence (Satta et al., 1995).

We choose to analyse the elastin organisation in different tortuous arteries according the presence of intimal thickening correlated to the elastin quantification in medial and intimal layers. Our results were significant in several ways. The selected arteries presented a considerable tortuousness (Adair et al., 1994). We have observed that the elastin concentration, measured by quantitation color-images, in the medial and intimal layer are similar in all cases, without statistical differences. The elastin organisation as evaluated by morphometric index such as ITI and EI. Our results confirm two differents groups depending of anatomical origin (cranial and abdominal territories) according to the same concentration of elastin. We expected to obtain a lineal correlation between the concentration of elastin and the increased or lower presence of intimal disease. As we were unable to re-establish this relation, there may be other factors.

We do not attributed this disparity to the sampling heterogeneity because in the criteria of inclusion we have taken into account factors such as age, arterial disease or personal history suggesting interference. Other research studies verified that the computer program used in our study was appropriate for measuring surfaces, contours and color-imaging analysis of histologic stains.

In our study, the abdominal arteries presented more elastin disorganisation and intimal thickenings that the cranial arteries for a similar elastin concentration. We can deduce that the morphometric and quantitation analyse must be associated with the others qualitative elements when we studied the arterial wall. Thus, we completed and specified the morphometric data and elastin concentration with other aspects which we believe conditioned the results.

The histological peculiarities described for each arterial group concerning the elastin distribution and tridimensional structure in the medial layer and the IEL integrity should be considered. The role of microfibrils in the arterial wall is important that the microfibrils have the capacity to change the orientation of the elastic fibers (Lillie et al. 1998). The degradation and disorganization of the elastin seem to be crucial in the formation of intimal thickenings (Ito et al., 1998). In the arteries considered, the levels of elastolysis varied depending on the territory and directly fostered intimal thickenings. We found a significant direct correlation between EI and ITI. This fact confirms the findings of Ross (1986), Fuster et al. (1995) and Libby (1995) for the pathogeny of atherosclerose.

The production of enzymes which degrade the extracellular matrix plays an important role in remodelling tissues. Currently it is believed metalloproteinases (Mmps) favor SMC proliferation and that they are involved in the formation

of atheromatose plaque (Zempo et al., 1994; Patel et al., 1996). Moreover, the role of Mmps is similar to the elastase (disorganisation and destructuration of elastin) and their production is associated with the SMC dedifferentiation state (Sasaguri et al., 1994). Loss of medial functional elasticity increases luminal wall stres, increasing the possibility of endotelial damage and predisposition to atherosclerosis (Avolio et al., 1998).

Finally, the hemodynamic changes, for which Levy et al. (1994) and Safar et al. (1996) have confirmed different distributions and concentrations of elastin and collagen in different arterial regions in essential arterial hypertension. The blood flow and the collaterally degree may not be the same in the abdominal and cranial regions, which show clearly different values for ITI and the EI.

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