

INVESTIGATIONS INTO HUMAN TRACHEAL CARTILAGE OSSEOCALCINEUS METAPLASIA IV. MORPHOKINESIS OF TRACHEAL CARTILAGE RETROGRADE LESIONS DURING THE PROCESS OF AGING

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We determined the frequency of occurrence and dynamism of the mentioned retrograde lesions. The investigated material comprised 371 cartilages collected from 95 male tracheas (mean age: 56 ± 13 years), and 279 cartilages collected from 70 female tracheas (mean age: 65.3 ± 14 years) during the process of aging. The dynamism proved non-homogenous with a visible gender difference. The empirical regression curves often crossed each other. Some of the presented curves in female patients were observed beginning two decades after that of male patients, and at lower levels. Thus, it seems hard to conclude that some processes considering tracheal cartilage morphokinesis always precede others.

Key words: trachea, cartilages, morphokinesis, aging.

Introduction

Few investigations exist concerning the process of aging of tracheal cartilages. Some publications demonstrated that the above-mentioned cartilages can undergo the process of ossification [1, 2], calcification [3] or fibrosis [4]. Osteocalcine remodelling of the cartilages begins in the third decade of life in male, and in the fifth decade of life in female patients [5]. According to some authors [6, 7], the development of retrograde tracheal cartilage lesions can be attributed to the disproportion between the size of the nutritive surface of the cartilage and its thickness. This leads towards malnutrition, and development of retrograde lesions, especially in the central segments of the cartilages. Our previous investigations [8] demonstrated a statistically positive correlation between tracheal thickness and patient age, both in the case of men and women. Autopsy investigations demonstrated no evident correlation between the retrograde tracheal cartilage lesions, and chronic morphological lesions present in other organs [9]. Linzbach and Gläser suggested that the above-men-

tioned lesions occurred more often in patients with chronic lung disorders, diabetes mellitus, and those who died due to cachexia [6, 10]. Siebenmann [11] demonstrated massive osseous metaplasia of the auricular cartilages in a patient undergoing chronic therapy, due to tuberculous spondylitis. Gläser [10] observed tracheal cartilage retrograde lesions with the development of asbestoids in patients of thirty years of age. On the contrary, Leutert [12] observed the above-mentioned in elderly patients and quite rarely. According to Linzbach [6], osseous lesions of the tracheal cartilages occurred more often in male patients, while calcifications in female patients. Kasafuka *et al.* observed a significantly increased percentage of tracheal cartilage osseous metaplasia in elderly patients [13]. Available literature data mentioned no tracheal cartilage retrograde lesion dynamics during the process of aging.

Material and methods

The investigated material comprised 371 cartilages collected from 95 male tracheas ranging between 19

and 84 years (mean age: $\bar{x} = 56 \pm 13$ years), and 279 cartilages collected from 70 female tracheas ranging between 18 and 90 years (mean age: $\bar{x} = 65.3 \pm 14$ years) ($p < 0.001$). Typical histopathological, 5 μm thick samples were prepared and stained in haematoxylin and eosin. Six types of cartilages were distinguished: 1- normal cartilage, 2-presence of mucous lakes and asbestoids, 3-eosinophilic staining with chondrocyte disintegration and precipitation of mineral salt dust, 4-massive mineral salt deposits, 5-coexistence of massive mineral deposits and osseous metaplastic foci, 6-osseous metaplasia. Tables present numerical and percentage data of the above-mentioned types of cartilages. The peak occurrence of each given type of cartilage lesion was estimated on the basis of empirical regression curves.

Results

Tables and Figures 1 and 2 demonstrate the dynamism of tracheal cartilage changes on the basis of empirical regression curves, considering both genders. The differences are especially visible in the case of comparison of most mild and severe lesions (types 3 and 6). The curve concerning type 3 changes in female patients is at its highest level, being stable throughout the entire life. In the case of male patients the highest peak is observed in the fourth decade of life, decreasing to 50% in the ninth decade of life, amounting to only 75% of values observed in the case of women. The curve in the case of osseous metaplasia (type 6) is completely different. In the case of male patients the curve begins in the fifth decade of life at values amounting to 22.5%, gradually reaching peak values (33%) in the eighth decade of life. In case of female patients isolated osseous metaplasia foci appeared in the sixth decade of life. The curve was observed at a three-times lower level than that of male patients, reaching zero values in the eighth decade of life, and increasing to 7.7% in the ninth decade of life. However, the value of 16.7% observed in male patients was never attained. Type 2 lesion differences were also observed, depending on the gender. Considering both sexes, mucous lakes and asbestoids appeared in the fourth decade of life. In the case of men, the above-mentioned curve gradually increased to maximum values of 33.3% in the ninth decade of life. In the case of female patients the curve was initially observed at levels of 8.3% in the fourth decade of life, reaching values of 5.8% in the ninth decade of life. We also observed differences in the dynamism of occurrence of massive calcium deposits, between genders. In the case of male patients the above-mentioned were observed in the third decade of life (4.2%) increasing to

Table I. The occurrence of the six different types of tracheal cartilage retrograde lesions in male patients, taking into consideration the different decades of life

CARTILAGE TYPE	18-30		31-40		41-50		51-60		61-70		71-80		81-90		SUMMARY	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1	4	16.67	0	0.00	0	0.00	3	3.57	2	2.41	0	0.00	0	0.00	9	2.43
2	0	0.00	2	7.40	4	5.00	7	8.33	10	12.05	19	28.36	2	33.33	44	11.86
3	16	66.67	22	81.48	36	45.00	33	39.29	32	38.55	19	28.36	3	50.00	161	43.40
4	1	4.17	1	3.70	14	17.50	17	20.24	10	12.05	6	8.96	0	0.00	49	13.20
5	3	12.50	2	7.40	8	10.00	12	14.29	6	7.23	1	1.49	0	0.00	32	8.63
6	0	0.00	0	0.00	18	22.50	12	14.29	23	27.71	22	32.84	1	16.67	76	20.49
Total	24	100.01	27	99.98	80	100.00	84	100.01	83	100.00	67	100.01	6	100.00	371	100.00

Explanations: N – number of investigated cartilages, % – percent of investigated cartilages, type 1 – normal cartilage, type 2 – chondrolysis and asbestoids, type 3 – eosinophilic staining of the cartilage, type 4 – massive calcium deposits, type 5 – mixture of calcineous foci and osseous metaplasia, type 6 – metaplasia ossea only

Table II. The occurrence of the six different types of tracheal cartilage retrograde lesions in female patients, taking into consideration the different decades of life

CARTILAGE TYPE	18-30		31-40		41-50		51-60		61-70		71-80		81-90		SUMMARY	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
1	4	33.33	5	41.67	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	9	3.23
2	0	0.00	1	8.33	0	0.00	1	2.27	1	1.67	2	2.78	3	5.77	8	2.87
3	8	66.67	6	50.00	21	77.78	31	70.45	49	81.67	60	83.33	39	75.00	214	76.70
4	0	0.00	0	0.00	4	14.81	3	6.82	6	10.00	8	11.11	6	11.54	27	9.68
5	0	0.00	0	0.00	2	7.40	5	11.36	2	3.33	2	2.78	0	0.00	11	3.94
6	0	0.00	0	0.00	0	0.00	4	9.09	2	3.33	0	0.00	4	7.69	10	3.58
Total	12	100.00	12	100.00	27	99.99	44	99.99	60	100.00	72	100.00	52	99.98	279	100.00

Explanations: N – number of investigated cartilages, % – percent of investigated cartilages, type 1 – normal cartilage, type 2 – chondrolysis and asbestoids, type 3 – eosinophilic staining of the cartilage, type 4 – massive calcium deposits, type 5 – mixture of calcinous foci and osseous metaplasia, type 6 – metaplasia ossea only

20.2% in the sixth decade, and diminishing to 0% in the ninth decade of life. In female patients the curve was observed in the fifth decade of life (14.8%) gradually decreasing to 11.5% in the ninth decade of life. The curve, considering the coexistence of calcium deposits and osseous metaplasia foci in tracheal cartilages, began in male patients at a level of 12.5% in the third decade of life, reaching maximum values of 14.3% in the sixth decade of life, followed by a systematic decrease to zero values in the ninth decade of life. In female patients the above-mentioned is as follows: begins in the fifth decade of life, reaching its peak (11.4%) in the sixth, and decreasing to zero values in the ninth decade of life.

In the case of male osseous metaplasia two peaks are observed. The first peak is observed in the fifth decade of life (22.5%), while the second (32.5%) in the eighth decade of life. In female patients the above-mentioned foci appear in the sixth decade of life (9%), decreasing to zero values in the eighth decade, and increasing once again to 7.7% in the ninth decade of life.

Discussion

There are age-related changes in the biomechanical properties and biochemical composition of airway cartilages that could influence airway dynamics [14]. After sexual maturity the transverse cross-section of the trachea increases, especially in male patients, its volume amounting to 40 ml, while that of female patients amounts to 20 ml [15].

The mean surface of male transverse cross-section tracheal cartilages amounts to 6 mm², while that of female cartilages – 4 mm² [6]. Considering men the above-mentioned hinders the diffusion of nutritional substances to the central parts of the cartilages. With age one can observe an increased cartilage resistance index, especially in its central parts [7]. The above-mentioned features favour the development of retrograde lesions.

Considering the ultrastructural image the tracheal cartilage stroma is viewed as a “honey comb”, built from collagen fibres, which are joined by mucopolysaccharide acids. During the process of aging these acids are subjected to shortening and thickening [16, 17]. The disappearance of mucopolysaccharide acids, hydroxyproline and water, as well as increase in the value of chondroitin-6-sulphate is the reason for the loss of metachromasia and development of an eosinophil cartilage reaction [10, 14, 18-20]. Tensile stiffness correlated inversely with the water and hydroxyproline contents [14].

Considering the cartilages of elderly patients, type II collagen diffusion staining is observed in the interchondrial matrix of all parts of the cartilages, while type I collagen only in the perichondrium. Type X

collagen is observed around the lacunae and developing osseous metaplasia [13].

The chondrocytes which are initially overgrown undergo degradation and atrophy. This favours the precipitation of mineral salts [7, 21], which leads towards cartilage rupture, and penetration of vascular fascicles into the fissures, inducing the development of osseous metaplasia foci, as an attempt to repair the damaged cartilage [6].

Investigations concerning the intervertebral disc cartilages show an overall incidence of SA- β -gal positive cells (senescence associated β -galactosidase) amounting to $29.9\% \pm 24$ SD [22]. The average length of telomeres decreased with the age of chondrocytes, especially in the cartilages of degenerative discs [23]. Such investigations considering tracheal cartilages are absent.

The presented study demonstrated the existence of evident differences in the dynamism of tracheal cartilage retrograde lesions between female and male patients. In the case of female patients, type 3 lesions predominated. In male patients, type 6 lesions were most often diagnosed (type 3 changes decreased since the fourth decade of life). The dynamism of chondrolytic lesions and asbestoids development was significant in male patients, beginning from the fourth decade of life, continuously increasing until the ninth decade. In female patients on the contrary, the above-mentioned was maintained at a low level. Gläser observed these lesions in patients of 30 years of age, being observed less frequently during subsequent decades of life [10]. Leutert rarely observed the above-mentioned, and if present they concerned the elderly only [12]. The development of asbestoids is connected with the degradation of collagen fibres, which was visible following Picrosirius staining [24].

Ultrastructural investigations showed an overall increase of collagen fibril diameter with increasing age, even in areas with no signs of amantoid transformation. Extensive remodelling not only of the collagen fibrils but also of the cartilage proteoglycans is involved in the development of amantoid transformation [25]. With increasing age, proteoglycan aggregates become shorter and fewer monomers were present in "Y-mice", since the sixth month of life [19], which transposed to patient age, would be around twenty years. Such developing cartilage and chondrocyte stroma lesions favour the precipitation of calcium deposits.

Our investigations showed that the calcium deposit curve in men began in the third decade of life, increasing to 20.2%, being stable throughout the fifth and sixth decades of life. In female patients the above-mentioned appeared twenty years later with the peak amounting to 15% in the fifth decade of life. Gläser observed cartilage calcification in 54% and ossification in 20% of investigated tracheas [10],

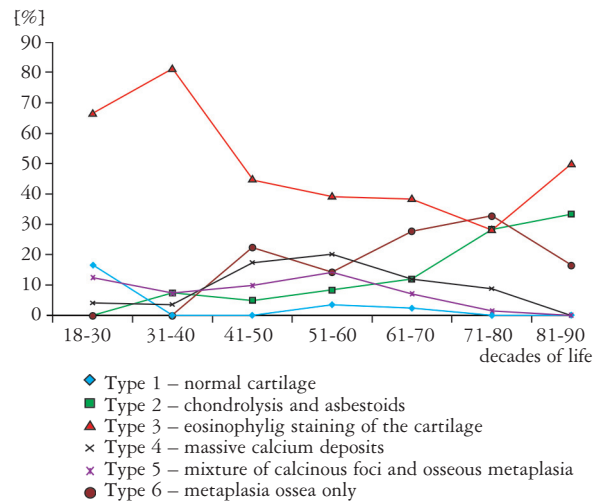


Fig. 1. Empirical regression of the fraction (%) of a given type of changes in tracheal cartilage in male

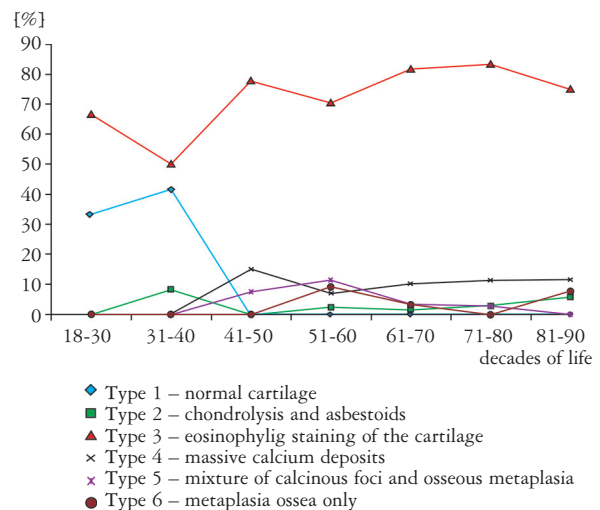


Fig. 2. Empirical regression of the fraction (%) of a given type of changes in tracheal cartilage in female

while Linzbach noted cartilage calcification mostly in female patients, being more often diagnosed as compared to osseous metaplasia foci [6]. The development of mineral deposits in tracheal cartilages is of different character, as compared to osseous calcification, where alkaline phosphatase is probably required [26]. Kasafuka *et al.* found ossification of investigated tracheas in 52% [13]. Fischer analysed chest X-rays demonstrating cartilage calcification and ossification in 9.5% of elderly female and only in 0.3% of male patients [27]. Bearing in mind the mechanism of bone development one should consider the degradation of the interchondrial matrix and deregulation of the function of perichondrium cells expressed by means of the bone morphogenic protein (BMP-6), which may play an important role in the maintenance of the tracheal cartilage by an autocrine mechanism [13]. Wang *et al.* [28] administered BMP-6 to an experi-

mental animal, which leads towards the development of an ectopic cartilaginous tissue.

The presented cartilage changes may be responsible for tracheal wall resistance. The average value for tensile stiffness ranged between 1 and 15 MP and increased significantly with age. The outermost layer of the cartilage was most stiff in all individuals, and thus, the deeper layers were progressively less stiff [14]. This might be connected with the localization of the previously mentioned lesions [9].

In conclusion, our investigations demonstrated unknown tracheal cartilage retrograde changes observed in the aging organism. The dynamism of selected changes was non-uniform and non-continuous, differing during different decades of life. Gender differences were also clearly visible [5, 9]. The mentioned curves often crossed. In the third decade of life one observed cartilage stroma staining changes, while mucous lakes and asbestoids were observed in the fourth decade of life. The curve considering mineral deposits and osseous metaplasia foci was shifted to the right by two decades in the case of female patients, as compared to men. Additionally, in the case of female patients (excluding type 3 changes) all were below the level of 15%, while in the case of male patients the curves were observed at a higher level with types 2 and 6 exceeding 30%.

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