Investigations into human tracheal cartilage osseocalcineus metaplasia II. Histopathological examination of tracheal cartilages

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Different opinions in the literature about changes in tracheal cartilages were the basis for investigations concerning the types of morphological changes occurring in ageing tracheal cartilages.
5 micron thick specimens stained with haematoxylin-eosin and in selected cases using von Kossa method of 371 cartilages taken from 95 male (mean age 56.6 ±13.4 years) and 279 cartilages from 70 female patients (mean age 64.9 ±16 years) ($p < 0.001$) were the investigated material.
The analysis demonstrated statistically significant cartilage type differences between men and women ($p < 0.001$). Chondrolysis and asbestoids were observed in 11.9% of male and 2.9% of female patients. Calcium deposits were seen in 13.2% of male and 9.7% of female patients, while cartilage ossification in 20.5% and 3.6%, respectively. The coexistence of calcium deposits and osseous metaplasia was observed in 8.6% of male and 3.9% of female patients.

Key words: tracheal cartilage, metaplasia, ossification.

Introduction

The trachea is composed of 16 to 20 hyaline cartilages. Until the age of 20 years its length increases to 11 cm. The number of cartilages remains unchanged; they only get thicker and larger [1-3]. The intercellular matrix is composed of type II collagen and proteoglycans [4]. In younger patients the matrix is of a basophilic character with a positive metachromatism, which is the evidence of the presence of chondroitin sulphuric acid [5, 6]. With age one can observe a chondroitin sulphuric acid reduction and an increased amount of keratin sulphuric acid. This leads towards the development of eosinophilic reaction. The number of chondrocytes is reduced [2]. One can observe the accumulation of intracellular glycogen and lipid deposits [5, 7]. Their protein-granular degradation leads towards the development of chondrolytic lesions and asbestoids [2, 3, 6], also called asbestoid or amianthoid degeneration [8, 9]. Gläser [6] observed the above in a 30-year-old patient, while according to Leutert [10], they rarely occurred being diagnosed only in elderly patients. With age patients are subjected to precipitation of larger calcium deposits and cartilage ossification [11, 12].

Well-known human anatomy textbooks [13, 14] do not mention the lesions observed in ageing tracheal cartilages. According to Marciniak, cartilage ossification begins at the age of 30 years [15]. According to Bochenek and Reichert [16], tracheal cartilages are subjected to ossification in elderly patients. Shah only mentioned that "they may become calcified in the elderly" [17]. Bloom and Fawcet deny that such cartilages are subject to ossification; they only undergo fibrosis [18]. Significant changes in the ageing trachea were diagnosed by means of radiological examinations in 66.4% of male and 33.3% of female patients [19]. Since the authors were unable to determine their anatomical substrate, and due to the above textbook differences [15-17] the term "osseocalcineus metaplasia" was used [19].

The textbook of Pathological Anatomy written by Henke-Lubarsch [20] mentioned only the fact of tracheal cartilage ossification, however, without considering the details concerning the occurrence and influence of gender. Thus, we decided to determine the type and occurrence of the above-mentioned lesions, considering sex, age and topography, as well as their relation with the main disease entity of the deceased.

Material and methods

The study material comprised 95 male tracheas, patient age ranging between 19 and 84 years ($\bar{x} = 56.6 \pm 13.4$ years), as well as 70 female tracheas, patient age ranging between 18 and 90 years ($\bar{x} = 64.96 \pm 15.98$ years) ($p < 0.001$). Transtracheal samples were randomly excised every 4 cm, beginning from the first cartilage. They were fixed in 10% formalin, embedded routinely in paraffin wax, sectioned horizontally at 5 μm thick samples, stained with HE, and in some cases with von Kossa method. In total, 371 cartilages from 95 male tracheas and 279 cartilages from 70 female tracheas, were analysed. For better visualization, 6 types of cartilages were distinguished:

- normal cartilage with basophilic stroma,
- chondrolysis and asbestoid foci (Fig. 1, 2),
- eosinophilic staining of the stroma of the cartilage with chondrocytic and stromal disintegration, and calcium dust deposition (Fig. 3, 4),
- extensive calcification deposits (Fig. 5),
- coexistence of calcium deposits and osseous metaplasia foci,
- osseous metaplasia of the cartilage (Fig. 6).

The analysis comprised the occurrence of a given type of the lesion, depending on the age and sex of the deceased patient. We also considered the extent of tracheal lesions and the correlation of cartilage lesions with the underlying disease responsible for the patient's death. χ^2 and t-Student tests were used

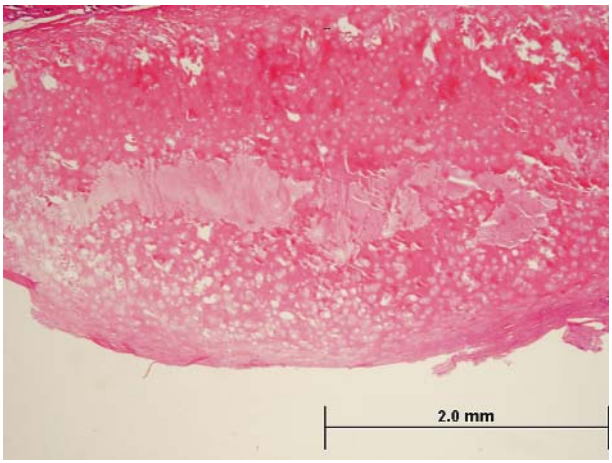

Fig. 1. Large chondrolysis ponds in the middle of the cartilage. HE, low-grade magnification

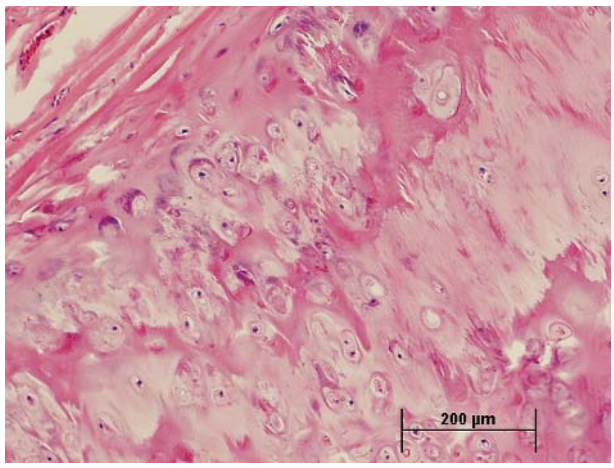

Fig. 2. Eosinophilic staining of the cartilage interstitium with chondrocytic destruction and multiple asbestoids. HE, medium-power magnification

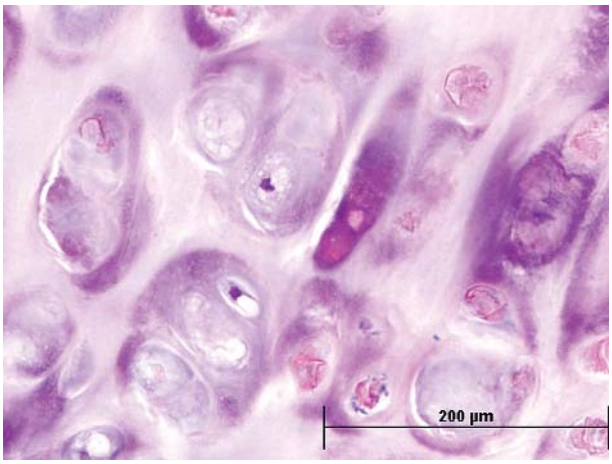

Fig. 3. Chondrocytic disintegration of the tracheal cartilage. HE, high-power magnification

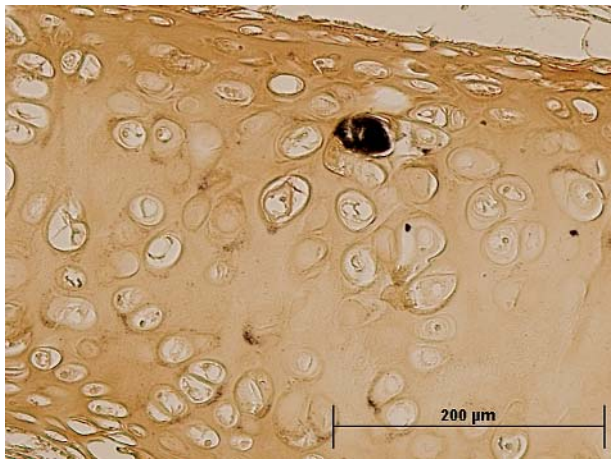

Fig. 4. Calcium dust of the interstitium and chondrocytes. Von Kossa, medium-power magnification

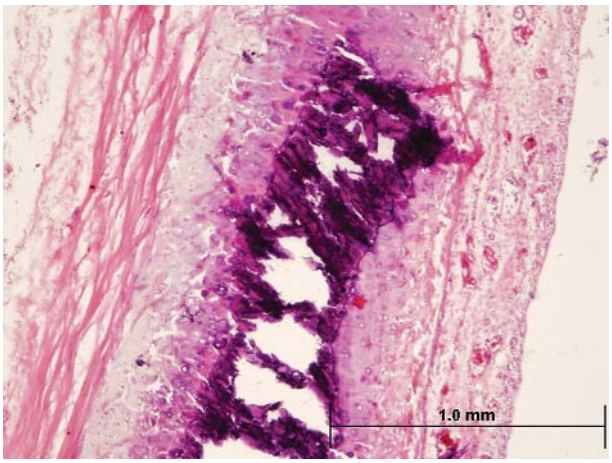

Fig. 5. Massive calcium deposits in the middle of the tracheal cartilage. HE, low-power magnification

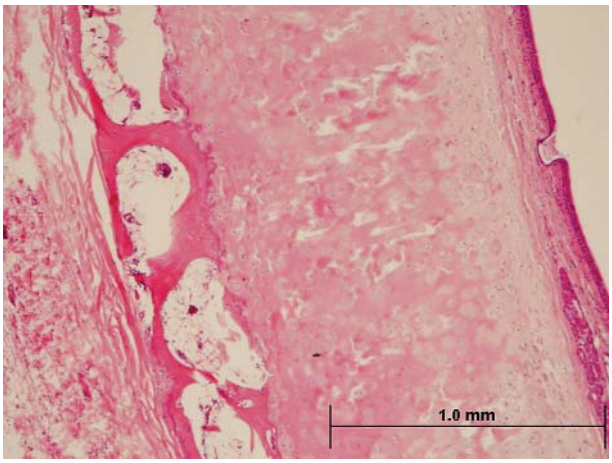

Fig. 6. Osseous metaplasia on the convex side of the cartilage. Tracheal mucosa on the right side. HE, low-power magnification

for statistical analysis. $P < 0.05$ was considered as statistically significant differences.

Results

The analysis showed significant differences, considering the occurrence of the above-mentioned types of lesions, depending on patient gender ($p < 0.001$). In female patients less important lesions predominated as compared to the male population. Type 3 was diagnosed twice as often in the female population, while chondrolysis and asbestoids were diagnosed four times more often in the case of male patients. Calcium deposits were observed in 13.2% of male and 9.7% of female patients' cartilages, while osseous metaplasia foci were diagnosed five times more often in male cartilages (Table I). The above-mentioned changes were mostly localized on the convex margin of the cartilage. Considering male patients only in one case was the lesion diagnosed on the concave margin of it, nearly above the mucous membrane, which amounted to 0.27% of 371 cartilages analysed. In relation to 108 cartilages with osseous metaplasia these figures amounted to 0.9%. Similarly, only one female patient was diagnosed with submucous metaplasia localized on the concave margin of the cartilage, which amounted to 0.36% of 279 analysed cartilages. In relation to 21 cartilages with osseous metaplasia these figures amounted to 4.76%, which is five times more than in the male group.

The coexistence of mineral deposits and osseous metaplasia foci was observed twice as often in male (8.63%) than female patients (4%). Type 2 cartilages in men coexisted with calcium deposits and osseous metaplasia foci in 32% of cases. The above-mentioned type was diagnosed in 50% of female patients. The third type of cartilages, which were diagnosed in 65.5% of the male population, was accompanied by chondrolysis, calcium deposits, and osseous metaplasia foci in 33% of cases. Type 3 lesions were diagnosed in 82.44% of female patients, being only accompanied by the above-mentioned in 6.95% of cases.

The average female patient's age was significantly higher (64.8 $\pm$16 years), as compared to the male population (56.9 $\pm$14 years) ($p < 0.001$).

In isolated cartilages, the average age of types 3 and 4 was significantly higher as compared to the male population ($p < 0.001$ and $p < 0.02$, respectively) (Table II). The mean age of the remaining types of cartilages showed no statistical difference, considering male and female patients.

Considering particular patient age groups, the mean female age of healthy cartilages was significantly lower as compared to the mean age of the remaining cartilage lesions. In male patients, the mean age of healthy cartilages was also significantly lower as compared to the remaining cartilage types. Additionally, the mean age of type 2 cartilages was the highest and statistically different as compared to the mean age of type 3 ($p < 0.01$) and type 4 ($p < 0.05$) lesions (Table II).

We also determined the topography of the most advanced cartilage lesions (mineral deposits and ossification) (Table III). There were no significant gender differences ($p = 0.3$). However, in male patients significant differences were observed, considering the mentioned lesions, depending on the level of occurrence ($p < 0.001$). Similar correlations were not observed in the female population ($p = 0.2$). If only a few cartilages (25%) were changed, calcium deposits were most often diagnosed. The more extensive the tracheal lesions, the fewer cartilages with calcium deposits (men: two times; women: 1.7 times), which were replaced by osseous metaplasia foci (men: 1.9 times, women: 3.8 times). Additionally, if only several cartilages were changed, the upper tracheal

Table I. The occurrence of a given morphological type, considering 371 cartilages obtained from 95 adult male trachea and 279 cartilages from 70 adult female trachea

Type of chondral changes	Males cartilage changes	N	%	Females cartilage changes	N	%
1.	normal cartilage	9	2.43	normal cartilage	9	3.23
2.	asbestoids and chondrolysis (pure) plus Ca^{2+} = 3 (4.62%) plus $Ca^{2+}$and ossification = 2 (3.08%) plus ossification = 16 (24.62%) total = 65 (17.52% of the whole material) pure pattern = 44 (67.7%)	44	11.86	asbestoids and chondrolysis (pure) plus albuminoid degeneration = 7 (43.75%) plus ossification = 1 (6.25%) total = 16 (5.73% of the whole material) pure pattern = 8 (50%)	8	2.87
3.	eosinophilia and albuminoid degeneration plus chondrolysis = 17 (5.65%) plus Ca^{2+} = 40 (13.29%) plus ossification = 25 (8.30%) total = 243 (65.5% of the whole material) pure pattern = 161 (66.26%)	161	43.40	eosinophilia and albuminoid degeneration plus Ca^{2+} = 8 (3.48%) plus Ca^{2+} and ossification = 1 (0.43%) plus ossification = 7 (3.04%) total = 230 (82.44% of the whole material) pure pattern = 214 (93.04%)	214	76.70
4.	calcifications only	49	13.20	calcifications only	27	9.68
5.	calcifications and ossifications	32	8.63	calcifications and ossifications	11	3.94
6.	ossifications only	76	20.49	ossifications only	10	3.58

N – number of cartilages; % – percentage of cartilages

Table II. Age of the deceased patients depending on the type of cartilage lesions considering both sexes

Type of chondral changes	Males					Females				
	N	%	Age			N	%	Age		
			Min.	Max.	$\bar{x} \pm SD$			Min.	Max.	$\bar{x} \pm SD$
1.	9	2.43	26	66	37.0 ±20.7	9	3.23	18	39	30.0 ±8.83
2.	44	11.86	38	84	61.07 ±12.69	8	2.87	36	90	71.0 ±17.44
3.	162	43.67	21	84	55.0 ±13.65	214	76.70	22	90	66.33 ±15.34
4.	49	13.20	33	77	55.89 ±11.89	27	9.68	42	84	64.94 ±12.78
5.	31	8.36	26	76	53.74 ±11.14	11	3.94	45	79	60.86 ±11.71
6.	76	20.48	43	84	60.85 ±15.68	10	3.58	53	82	64.75 ±11.71
Total	371	100.00	21	84	56.89 ±14.02	279	100.00	18	90	64.76 ±16.0

N – number of cartilages; % – percentage of cartilages, min. – minimal age, max. – maximal age, X ±SD – average age plus/minus standard deviation

cartilages were considered. However, if the lesions extended to the lower trachea, all cartilages were similarly changed, ranging in men between 25.3% and 26.6%, and in women between 23.5% and 29.4%.

Table IV demonstrates that there was no correlation (apart from aortic atherosclerosis) between the occurrence of the above-mentioned tracheal cartilage lesions and the main disease of the deceased.

Discussion

Our investigation is the first attempt to statistically consider the above-mentioned issue. Results

Table III. Topography of tracheal lesions depending on the number of affected cartilages

Tracheas		Male				Female			
number of changed cartilages	number of the cartilage	Ca^{2+}	ossification	total	%	Ca^{2+}	ossification	total	%
25% changed cartilages	1	6	2	8	40.00	3	1	4	50.00
	2	4	4	8	40.00	3	0	3	37.50
	3	0	1	1	5.00	0	0	0	0.00
	4	2	1	3	15.00	1	0	1	12.50
	total	12 (60%)	8 (40%)	20	100.00	7 (87.5%)	1 (12.5%)	8	100.00
50% changed cartilages	1	8	10	18	40.00	3	1	4	36.36
	2	11	8	19	43.18	2	0	2	18.18
	3	3	0	3	6.82	2	1	3	27.27
	4	4	1	5	9.09	1	1	2	18.18
	total	25 (56.82%)	19 (43.18%)	44	100.00	8 (72.73%)	3 (27.27%)	11	100.00
75% changed cartilages	1	5	7	12	26.09	5	4	9	39.13
	2	7	9	16	34.78	4	4	8	34.78
	3	3	4	7	15.22	3	0	3	13.04
	4	8	3	11	23.91	2	1	3	13.04
	total	23 (50%)	23 (50%)	46	100.00	14 (60.87%)	9 (39.13%)	23	100.00
100% changed cartilages	1	5	15	20	25.32	2	2	4	23.53
	2	3	16	19	24.05	2	2	4	23.53
	3	5	14	19	24.05	2	2	4	23.53
	4	7	14	21	26.58	3	2	5	29.41
	total	20 (25.32)	59 (74.68%)	79	100.00	9 (52.94%)	8 (47.06%)	17	100.00

N – number of cartilages; % – percentage of cartilages

Table IV. The percentage of tracheas with documented lesions during the course of coexisting chronic diseases.

Item	Diseases	Male		Female	
		changed	unchanged	changed	unchanged
		tracheas (%)		tracheas (%)	
1.	diabetes mellitus	3.57	0.00	15.00	8.33
2.	liver cirrhosis	8.93	4.35	20.00	2.08
3.	cachexia	8.93	4.35	15.00	25.00
4.	pleural adhesions	23.21	26.09	30.00	35.42
5.	chronic pulmonary emphysema	50.00	43.48	45.00	25.00
6.	chronic lung tuberculosis	14.29	4.35	0.00	6.25
7.	aortic sclerosis	66.07	47.83	75.00	70.83
8.	heart defects	8.92	4.35	5.00	6.25
9.	malignant neoplasm and intracranial tumours	21.43	39.13	25.00	25.00
		} 33.93	} 52.17	} 25.00	} 29.17
10.	lymphoma and leukemia	12.50	13.04	0.00	4.17

pleural adhesions: single and multiple, aortic sclerosis: grade 1-3, heart defects: congenital and acquired, liver cirrhosis: alcoholic and postinflammatory.

demonstrated a significantly more frequent occurrence of mentioned lesions in male tracheal cartilages as compared to female cartilages. The mean age of male changed cartilages was ten years less as compared to that of female patients. If fewer cartilages with lesions were observed, more were diagnosed with calcifications. The more cartilages with lesions, the greater the predominance of osseous metaplasia foci.

Another interesting phenomenon was the localization of tracheal cartilages depending on the number of cartilages with lesions. In the case of fewer changed cartilages, lesions were localized in the upper part of the trachea. When lesions predominated, they were evenly distributed throughout the tracheal length.

Biochemical and microscopic results of the ageing tracheas, both in human beings [3, 6, 11, 21-23] and animals [5, 12, 24] are not homogeneous. During the process of tracheal cartilage calcification some authors did not observe proteoglycan depletion [12], while others noted its reduction [4]. Even when total tissue glycosaminoglycan and water levels were maintained, one observed increased tracheal cartilage proteolysis, which led towards a hydroxyproline reduction [23]. The amount of chondroitin sulphate decreases, while that of keratin sulphate increases, proportionally to the ageing of the cartilage [5]. Thus, the stromal environment becomes eosinophilic. Such changes were observed by Kasafuka *et al.* [11] in 84% of cases. Our own investigations confirmed the above, especially in female patients, being diagnosed twice as often as males. Chondrolysis and asbestoid foci were often diagnosed: four times more often in male patients as compared to the female population, even in the fourth decade of life. Gläser came to the same conclusions [6], being in opposition to results obtained by Leutert [10]. Asbestoid or amanthoid degeneration is the most common regressive change in the ageing hyaline cartilage matrix and deposition of parallel or radially disposed fibres, which lend to the tissue an appearance that resembles asbestos [8, 9]. Bonucci *et al.* [5] observed no such changes in experimental animals. With age the thickening collagen fibres continue to undergo calcification. Chondrocytes are also subjected to calcification, which in the absence of alkaline phosphatase activity should be considered as a normal ageing process, and is evidence of lack of natural ossification in the tracheal cartilages [12]. Considering our material, independently of the process of ossification, calcium stromal deposits occurred 1.5 times more often in male patients as compared to female patients, although in the latter, 2.5 times more often than osseous metaplasia foci.

Gläser showed calcium deposits in 54% of cases, mainly in the vicinity of osseous metaplasia foci development. No such impression was observed after the analysis of our material.

The occurrence of osseous metaplasia in tracheal cartilages was similar to that observed by Gläser [6]. Kasafuka *et al.* [11] demonstrated the above in 52% of patients, considering them as a physiological phenomenon connected with the ageing process of the cartilage. He observed no such lesions until the sixth decade of life, while in the seventh decade even 55.6% of tracheas presented with osseous metaplasia. The small material of investigated cases might pose doubts, considering the accuracy of obtained results.

Between the age of 60 and 80 years the male trachea is subjected to sword-like stenosis, while that of female patients to dilatation, which might be connected with the phenomenon of osseous metaplasia [1-3].

Considering the possible pathogenesis of the above lesions the following hypothesis was put forward: disproportion between the thickness of the cartilage and lack of efficient nutrient supply to its central parts. Measurements of the cross-section surface of the male cartilages (patients aged between 20 and 40 years) amounted to an average of 6 cm^2, while that of female patients only 3.6 cm^2 [3]. The above-mentioned difference might condition less frequent occurrence of these lesions in female tracheal cartilages. Newborn trachea lack ossification, calcification, type I and X collagen as well as type II collagen reduction [16]. Thus, the development of the above-mentioned lesions was probably connected with the disintegration of the cartilage, when during biomorphosis it attained its border-line thickness enabling nutrient supply [3].

Available medical literature showed no evidence of the protective effect of female sex hormones on the occurrence of the above-mentioned tracheal cartilage lesions. However, the phenomenon consisting in the inhibiting effect on the atheromatous development of sex hormones and negative influence of smoking is well-known in medicine. The above might be responsible for less frequent occurrence of these lesions in female tracheal cartilages.

Summarizing we may say that advanced retrograde tracheal cartilage lesions were observed significantly more often in men as compared to women. Ossification and calcification of cartilages occurred on average ten years later in female patients as compared to the male population. In the end we suggest that more important changes (calcifications and ossification), considering both genders, occurred independently.

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