FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 45, No. 3, 2007
pp. 221-227

Morphological aspect of voice disturbances of aged persons coexisting hypopharynx cancer

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Abstract: The voice quality in prebysphonia is conditioned by morphological changes in the vocal folds mucosa. The studies including light microscopy and transmission electron microscopy (TEM) revealed changes within the basal membrane epithelium and the stroma of the vocal folds mucosa. Age-related changes in thickness of the epithelium and direction of the basal membrane, increased number of collagenous fibres (C) and fibroblasts and chronic inflammatory process in the stroma were found. Vacuolated and keratinised epithelial cells, enlarged extracellular spaces and numerous blood vessels confirm the edematous form of prebysphonia. Thinned epithelium with signs of hyalinization, inflammatory infiltrations in the stroma with numerous collagenous fibres and small number of blood vessels indicate atrophy of the vocal folds mucosa. Edematous and atrophic changes in the vocal folds mucosa are most frequently reported form of prebysphonia.

Key words: Prebysphonia - Vox senium - Vocal folds atrophy - Larynx edema - Hypopharynx cancer - Dysphonia - Voice quality

Introduction

Ageing of a human being is a biological process and an unpreventable fate [17]. The senile age is related to a decreased efficiency of the organism [5,8,15,18]. The voice organ, as well as other elements of the human body, is submitted to the ageing process, which begins around the age of 60 and takes an individual course [15]. The voice disturbances in the elderly are named the senile voice, vox senium or prebysphonia. According to many authors prebysphonia is conditioned by involutional changes in CNS accompanied by endocrine, pulmonary and anatomical changes in the larynx and throat mucosa [1,15,19,20]. According to Bennett, Klajman and Morrison *et al.* [2,3,9,13] larynx changes in the senile age should be termed as the polypomatous degeneration or atrophy. Pruszewicz [14,15] claims that the senile hypokinematic dysphonia is caused by a decreased tension in the vocal folds which results in a hourglass-shaped phonatic insufficiency of the glottis due to a decreased tension of the vocal and the arytenoideus transversus muscles. The vocal folds oedema is known as the polypoid degeneration or the Reinke's edema. The condition is a particular variety of the chronic inflammation of the larynx which develops on the glottis level [4,12,19,20]. The etiology of the Reinke's edema arise from accumulation of a fluid in the supraepithelial space due to permeability of the blood vessels and formation of an exudate [4,10,11]. The most frequent incidence of the disease is in the IV decade of life and does not affect young people. According to Abrams, Pruszewicz and Zalesska-Kręcicka [1,15,19,20] a frequent larynx pathology in the elderly is a degenerative larynx inflammation which is accompanied by atrophy of the tunica muscularis, metaplasia of the epithelium, atrophy of the connective tissue and the glandular tissue. Morphological transformation in the larynx results in disturbances of the voice quality and many subjective complaints in the elderly.

The aim of this study was a morphological assessment of the vocal folds mucosa in the elderly coexisting hypoharynx cancer with the use of light microscopy and transmission electron microscopy.

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Table 1. Clinical characteristics of the morphological material.

Group		n	Sex		Total	Age	
		%	Females	Males		Age span	Average
Control		n	12	8	20	35-38	36
		%	60	40			
Analysed	Material After surgery	n	15	19	34	65-72	68
		%	44.1	55.9	68		
	Autopsy Material	n	13	3	16	65-74	69
		%	81.3	18.7	39.3		

Table 2. Clinical diagnostics of the base of morphological material in analysed group.

Pathology	Material After Surgery				Autopsy Material				Total	
	Sex				Sex					
	Females		Males		Females		Males			
	n	%	n	%	n	%	n	%	n	%
Vocal folds edema	13	39	5	14.7	9	56	1	6	28	56
Vocal folds atrophy	2	5.9	14	41	4	25	2	12	22	41

Materials and methods

The study samples for morphological studies were collected from the intermembranous part of the vocal folds mucosa. The control group consisted of 20 persons aged 35-38 (average 36) with a prevalence of women (60%). All specimens were collected during autopsy. In the group of the elderly 50 specimens were analyzed. The age span was 67-74 years (mean age 68.5) years. The group of 34 (68%) patients with the cancer of hypopharynx (piriform sinus carcinoma) at the age from 65 to 72 years old was included in the study. Epidemiological aspects were the criteria for selecting the patients. Only 4 (12%) patients were smokers, and 6 (18%) patients were exposed to plant pesticides. In the group there were 15 (44%) female and 19 (56%) male patients. In the study material 68% (n=34) specimens were collected as a result of the total laryngectomy due to the recessus piriformis carcinoma. The specimens were compared with the autopsy material obtained from 16 (39%) dead persons without oncologic anamnesis at the age from 65 to 74. In this group there were 13 (81%) women and 3 (19%) men and 2 (12%) of them were smokers.

For light microscopy, the study material was fixed in 10% formaldehyde solution and subsequently dehydrated in the ascending alcohol row. The specimens were mounted in paraffin blocks, dissected and H+E stained. For TEM, the vocal folds mucosa samples were fixed in 2.5% glutaric aldehyde in kakodylate buffer, pH=7.4 for 4 hrs, postfixed in 1% OsO_4 for 2 hrs and subsequently mounted in Epon 812. Half-thin specimens were stained with methylene blue and preliminarily evaluated with the use of light microscopy. Ultrathin specimens were cut on the Reichert Ultracuts microtome, contrasted with lead citrate and uranyl acetate and finally analysed with the use of the transmission electron microscope Opton 900 PC.

Results

In the control material the light microscopy studies revealed the multilayer flat epithelium alongside the vocal fold, with the multistratified ciliated epithelium in the area of the ventricle of larynx (Fig. 1)

In the analysed material the smooth surface of the mucosa, the keratinising paraepidermoid epithelium with enlarged blood vessels and infiltrations from plasma cells and lymphocytes under the epithelium were observed (Fig. 2)

In other specimens the thinned paraepidermoid epithelium and partly the multistratified epithelium were found. Under the epithelium small number of plasma cells and lymphocytes were observed (Fig. 3)

In the control group TEM specimens revealed the multilayer flat epithelium with folds in the basal membrane, blood vessels, numerous pericytes (P) and scanty collagenous fibres (C) in the stroma (Fig. 4).

In the analysed material signs of the epithelial cells destruction with an increased vacuolisation and enlarged intercellular spaces were found. In the stroma a large number of blood vessels, fibroblasts (F) with the signs of vacuolisation, collagenous fibres (C) and inflammatory cells were found. In branches of the fibroblasts (F) enlarged cisterns of the granular endoplasmic reticulum were evident. The folds of the epithelial basal membrane were less pronounced than

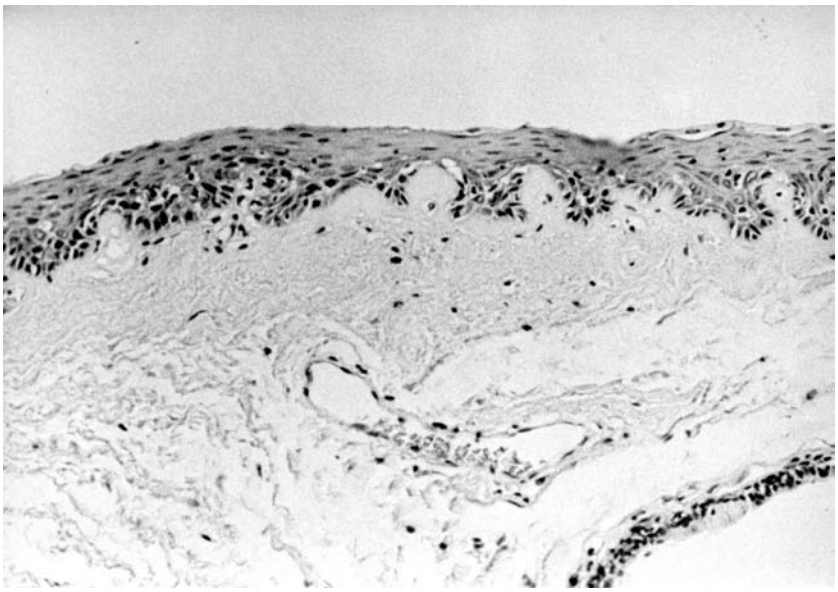

Fig. 1. The vocal fold mucosa in the control group (H+E, magnification ×180)

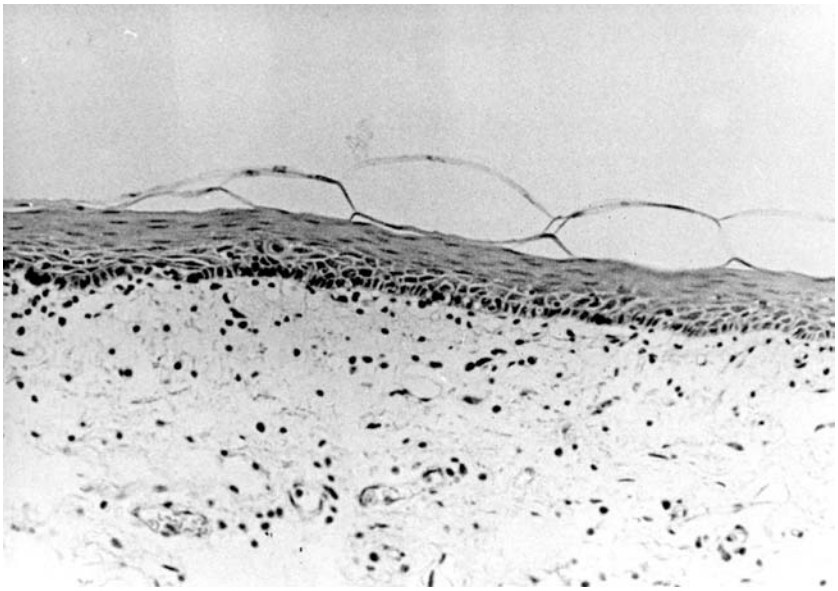

Fig. 2. The vocal fold mucosa with infiltrations from mononuclear cells (H+E, magnification ×180)

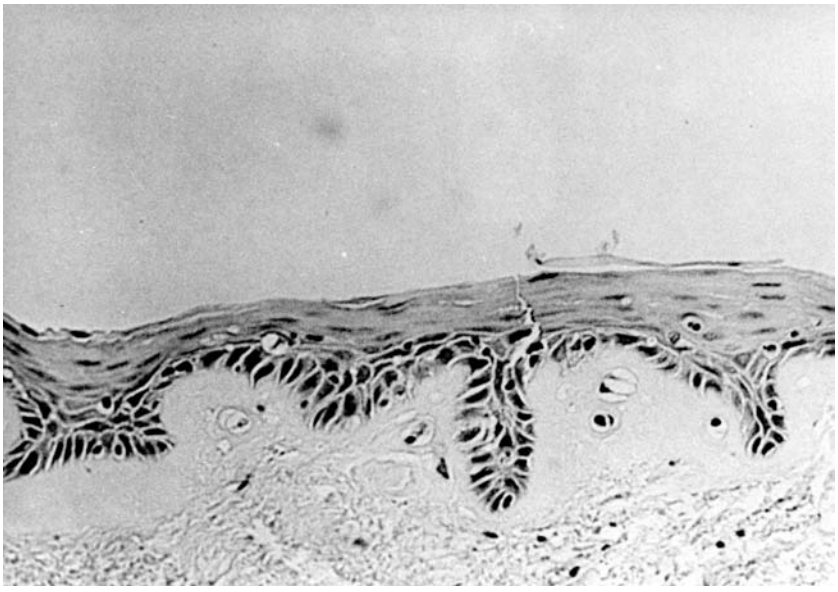

Fig. 3. Thinned epithelium of the vocal fold mucosa with small number of plasmatic cells and lymphocytes. (H+E, magnification ×180)

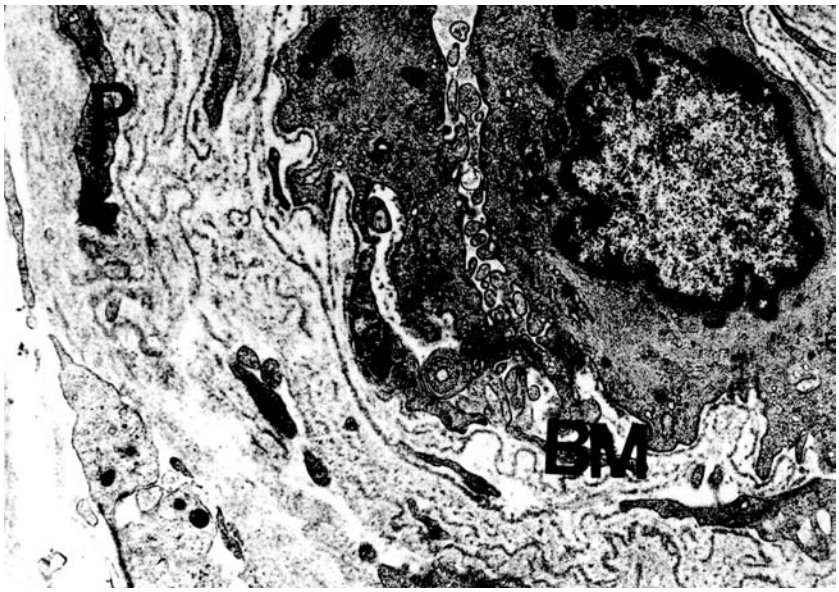

Fig. 4. The vocal fold mucosa in the control group. Fragment of the blood vessel. Numerous branches of pericytes (P) (TEM, magnification ×2700)

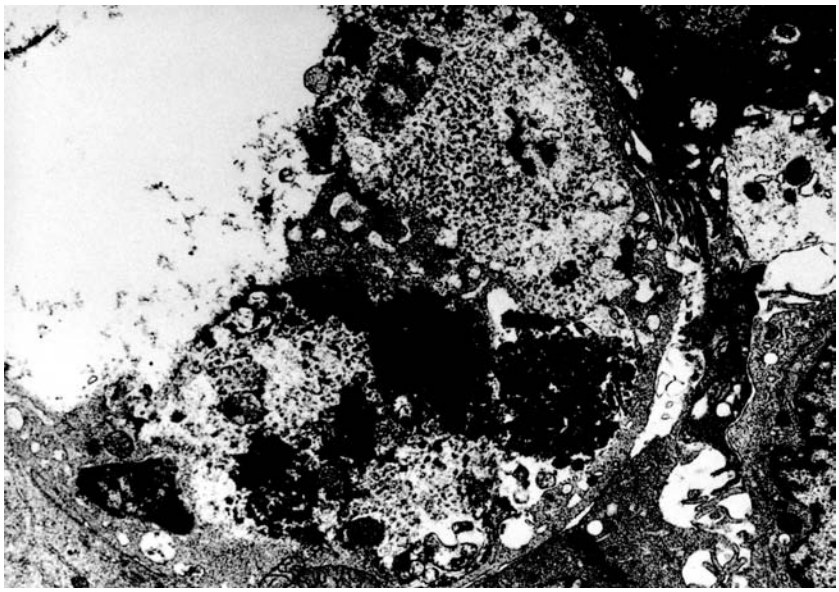

Fig. 5. Signs of the epithelial cells destruction with enhanced vacuolisation (TEM, magnification ×1800)

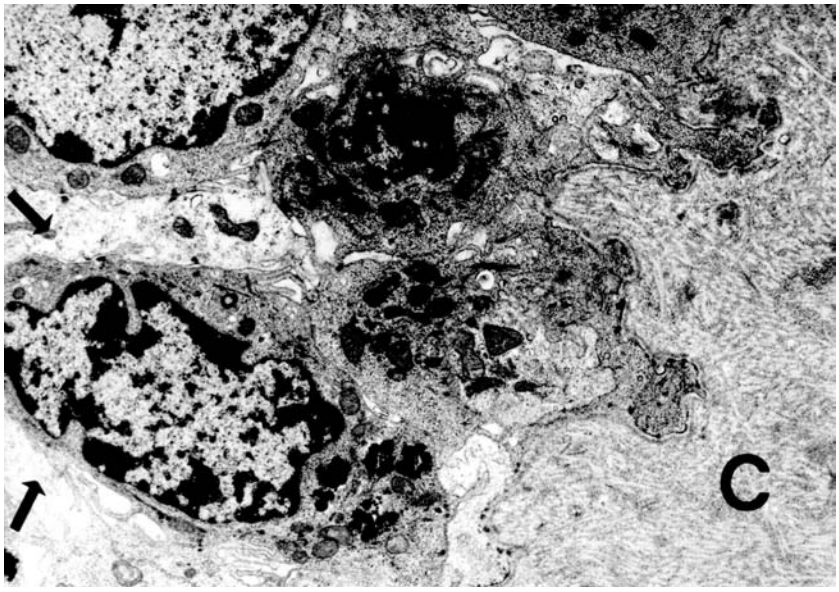

Fig. 6. Enlarged intercellular spaces in the epithelium (arrows). Numerous collagenous fibres (C) in the stroma (TEM, magnification ×1800)

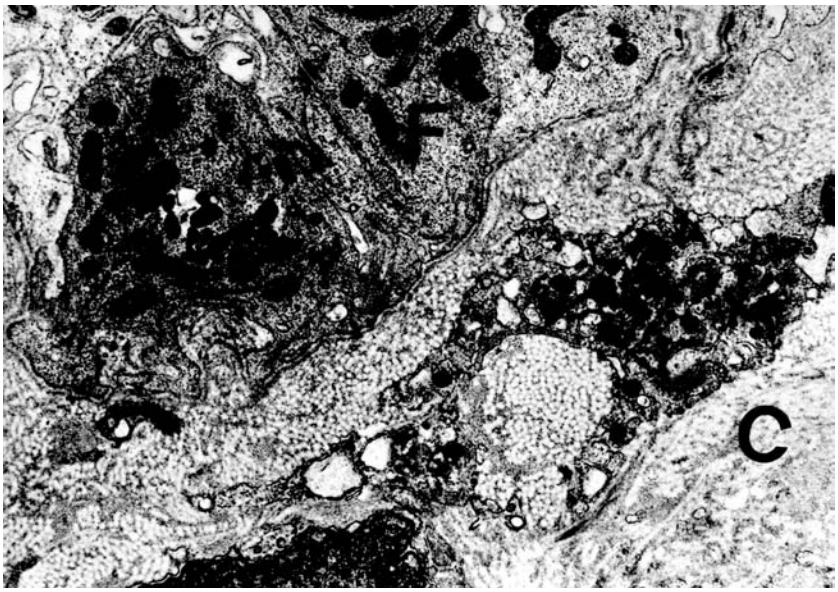

Fig. 7. Fibroblasts (F) and numerous collagen fibres (C). Enlarged cisterns of the granular reticulum in the branches of fibroblasts (TEM, magnification ×1800)

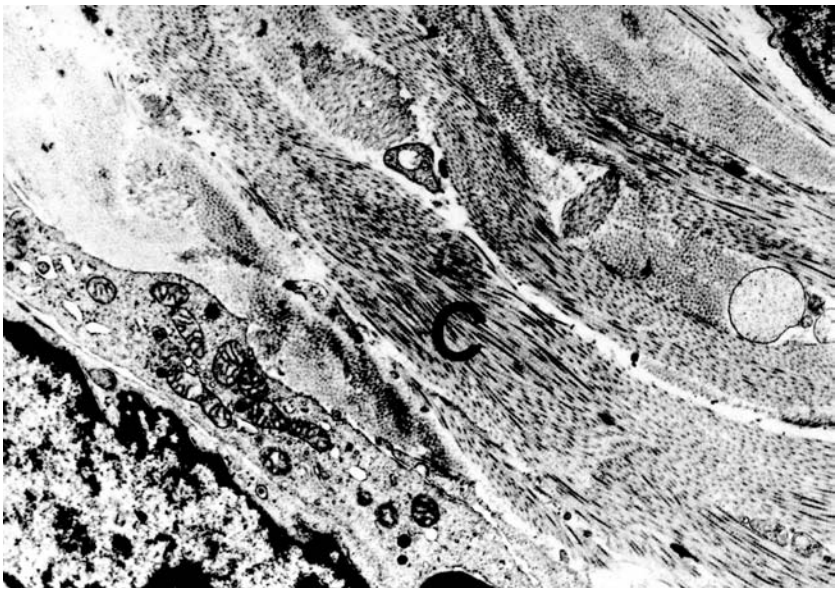

Fig. 8. Very numerous collagenous fibres (C) in the stroma (TEM, magnification ×1800)

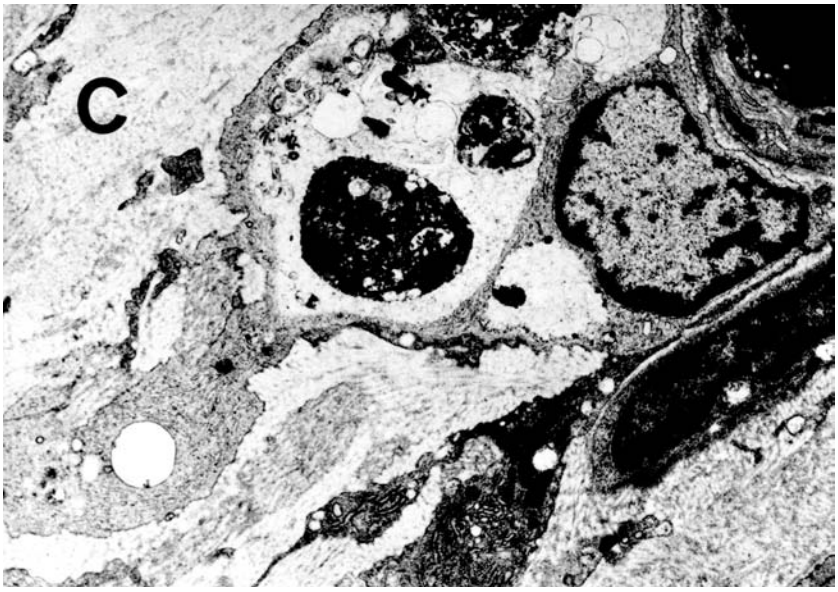

Fig. 9. Signs of destruction and degeneration of fibroblasts with large fagosomes in the cytoplasm. Numerous collagenous fibres (C). A blood vessel in the right upper corner (TEM, magnification ×1800)

those in the control group (Fig. 5, 6 and 7) and in many specimens the stroma contained large numbers of collagenous fibres (C), inflammatory cells and fibroblasts with traits of the vacuolic degeneration. Scanty blood vessels exhibited multiplications of the basal membranes (BM). The folds of the epithelial basal membrane were less pronounced than those in the control group (Fig. 8 and 9) and in 18 (52%) patients with the piriform sinus carcinoma we found coexistent oedema tic morphologic changes of vocal folds mucosa. In 16 (47%) patients atrophic changes were found. Oedema was found in 13 (39%) women and 5 (14%) men. Atrophy was found in 2 (6%) women and 14 (41%) men.

In the autopsy female material, edema was found in 9 (56%) and atrophy was found in 4 (25%) women. In male autopsy material atrophic changes were found in 2 (12.5%) men and edematic changes were found in 1 (6%) cases.

Discussion

Analysis of specimens of the vocal folds mucosa revealed influence of ageing on the membrane morphology. All the patients were classified as being of the senile age according to the WHO classification. The group of patients with hypopharynx cancer was the selected group with possibly the least significant influence of irritating agents. With the use of light microscopy differences with relation to the control group were found, which included keratinisation of the epithelium, infiltrations from plasma cells and lymphocytes and numerous enlarged blood vessels. According to Bętkowski, Rak, Zalesska-Kręcicka [4,6,19,20] keratinisation of the epithelium is characteristic for the Reinke's edema. In other specimens the paraepidermoid epithelium and partly the multilayer epithelium were thinned, moreover, signs of hyalinization and infiltrations from plasma cells and lymphocytes were found. Similar morphologic changes in patients with clinical signs of the senile atrophy, manifested by the flattened epithelium with inflammatory infiltrations in the stroma, were described by Lehmann *et al.* [12]. Ultrastructural studies revealed significant differences in the analysed groups. Signs of epithelial cells destruction with enhanced vacuolisation and enlarged intercellular spaces were found. Besides a large number of the blood vessels and the collagenous fibres, fibroblasts with signs of vacuolic degeneration were found in the stroma. In the branches of fibroblasts, enlarged cisterns of the granular endoplasmic reticulum were observed. According to Rak [16] ultrastructural changes in the cell cytoplasm include the endoplasmic reticulum, ribosomes and mitochondria. The vacuolic degeneration suggests the vocal folds edema of the Reinke's type [16]. Similar morphological changes in the vocal folds mucosa of patients with the larynx edema were described by many authors [2,3,6,7,9,13]. The thinned epithelium, small foldings of the basal membrane, numerous collagenous fibres and inflammation in the stroma confirmed the clinical form of the atrophy of the vocal folds mucosa. Similar morphological changes were described by Hirano, Kurita *et al.* [6,7,10,11] in patients with the vocal folds atrophy after the age of seventy. According to many authors, morphological transformation of the vocal folds mucosa result in disturbances of the voice quality in prebysphonia [6,7,15,19,20].

Morphologic analysis of the vocal folds mucosa indicates age - related differences not related with coexistence of cancer hypopharynx. The vacuolic degeneration of the epithelial cells with signs of keratinisation, enlarged intercellular spaces, fibroblasts with traits of vacuolic degeneration and bronches of them and enlarged cisterns of the granular endoplasmic reticulum indicate the vocal fold edema in the elderly patients. Thinned epithelium with signs of hyalinization, large number of collagenous fibers and scanty blood vessels with inflammation in the stroma indicate atrophy of the vocal folds mucosa in the senile age. The folds of the epithelial basal membrane were less pronounced in elderly patients. Edenatic changes of vocal folds are more common in elderly women however in elderly men atrophic ones are more common.

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Submitted: 22 November, 2006
Accepted after reviews: 13 April, 2007