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Histological and Electron Microscopic Investigation of Reinke's Edema

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Reinke's edema is a benign lesion of vocal fold affecting subepithelial space. This paper describes the histological features of Reinke's edema on the basis of an extensive number of cases (203 women and 58 men). In 10 cases the electron microscopic examination was performed. Edema of subepithelial tissue was present in 138(62%) cases. This phenomenon was observed more frequently in women than in men ($p=0.01$). In the subepithelial tissue there were a numerous wide vessels with oedematous endothelium. Leukoplakia and dysplasia of epithelium were present in 21(8%) and 16(6%) specimens, respectively. Leukoplakia was detected more often in men than in women. This relation was close to statistical significance ($p=0.055$). The presence of dysplastic lesions of the epithelium was correlated with the age of the patients and smoking habit ($p=0.0042$, $p=0.0021$). Electron microscopic investigations revealed loosened intercellular junctions and widening of intercellular spaces, especially in basal and spinous layers.

Introduction

Reinke's edema is a benign lesion of vocal fold affecting subepithelial space. The entire length of the membranous vocal fold is edematous. The most frequent etiologic factor of Reinke's edema is smoking. The mechanism of the onset and development of the disease remains unclear [12]. Although Reinke's edema is a common cause of substantial changes of voice quality in adults the number of publications on this subject is limited. The majority of the studies deal with all benign lesions of the vocal folds e.g. Reinke's edema, vocal nodules and polyps [2, 4, 11]. Furthermore, histological features of Reinke's edema were not entirely elucidated [4, 5, 15].

This paper describes the histological features of Reinke's edema on the basis of an extensive number of cases. Special attention was drawn to analysis of dysplastic changes of epithelium. Ultrastructural picture of the epithelium and subepithelial space obtained in electron microscopy was also presented.

Material and Methods

The study population consisted of 261 patients with Reinke's edema treated in the Department of Otolaryngology, Medical University of Wrocław between 1994 and 2000. There were 203 women and 58 men in this group.

The patients had their clinical diagnoses verified by an experienced laryngologist who evaluated them by means of videostroboscopy. In all cases endolaryngeal microsurgery was performed. The histological specimens were obtained from the Department of Pathomorphology, Medical University of Wrocław. In 10 cases the electron microscopic examination was performed in the Department of Histology, Medical University of Wrocław.

Surgical specimens were fixed in 2.5% glutaraldehyde at 4°C for 2 hours, rinsed with cacodylate buffer solution and postfixed in 2% osmium tetroxide at 4°C for 2 hours. These specimens were dehydrated in a graded series of ethanol and embedded in epoxy resin. Semithin section were prepared with an ultramicrotome, stained with 1% toluidine blue, and examined with a light microscope. Thin sections were made with an ultramicrotome and stained with uranyl acetate and lead citrate and tannic acid stain. A JEM 100 B transmission electron microscope was used for study.

Statistical analysis

Relations between the features were estimated using Chi-square test. The p value less than 0.5 was considered as statistically significant.

Results

The analysis of histopathological specimens of the patients with clinical diagnosis of Reinke's edema revealed laryngeal polyp in 29(13%) cases. Edema of subepithelial tissue was present in 138(62%) cases (Fig. 1). This phe-

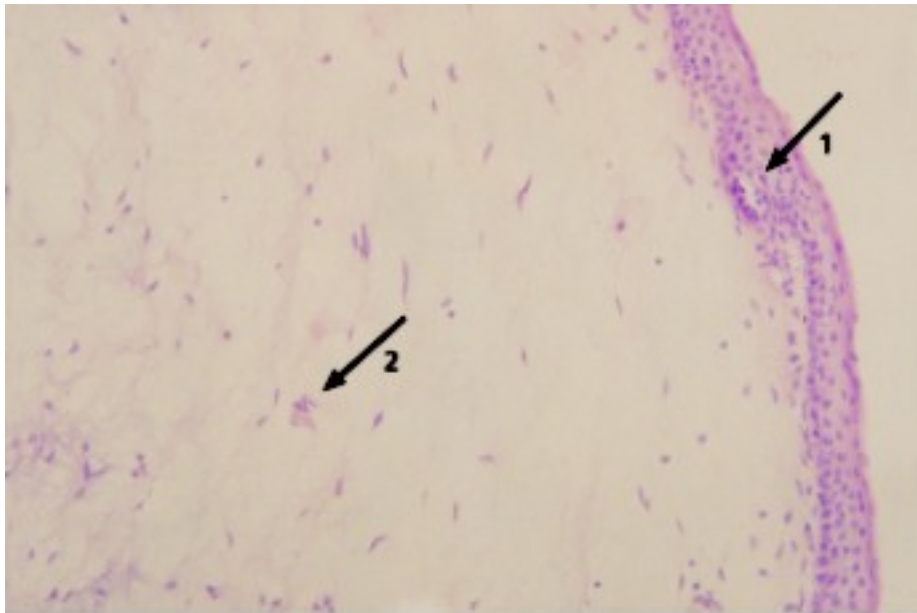


Fig. 1. Thin epithelium with normal cytoarchitecture (1). In subepithelial space substantial edema (2).

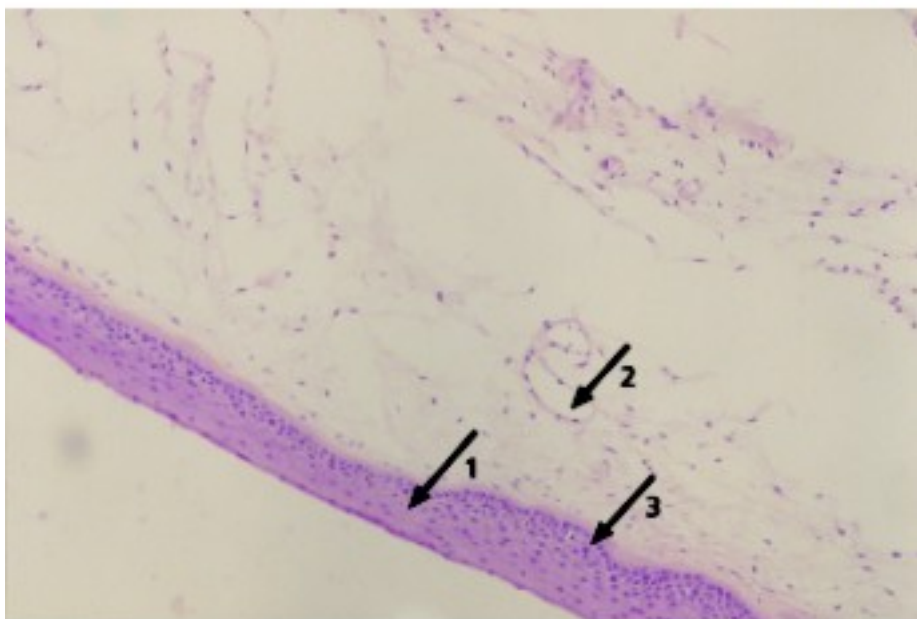


Fig. 2. Thin epithelium (1). Wide vessel in subepithelial space (2). Fibroelastin under basement membrane (3).

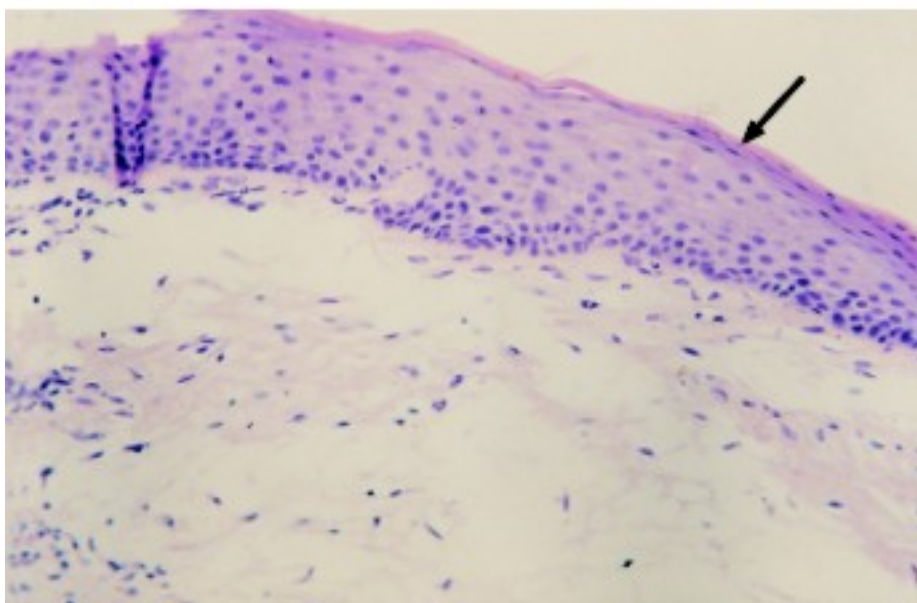


Fig. 3. Leukoplakia without dysplastic lesions of epithelium.

TABLE 1

Relation between sex of the patient and edema of the subepithelial tissue

Sex	Lack of the edema	Edema	p*
Women	72	131	0.01
Men	31	27	
Total	103	158	

p*- chi-square test

TABLE 3

Relation between age of the patient and dysplastic lesions of laryngeal epithelium

Age of the patient	Lack of dysplasia	Dysplastic lesions	p*
Under 40 years of age	30	2	0.004
Between 40 and 60 years of age	186	6	
Above 60 years of age	29	8	
Total	245	16	

p*- chi-square test

nomenon was observed more frequently in women than in men. This relation was statistically significant (Table 1) ($p=0.01$). The epithelium was usually thin and deposits of fibroelastin were visible under basement membrane.

In the subepithelial tissue there were numerous wide vessels with oedematous endothelium (Fig. 2). Histological signs of chronic laryngitis were detected in 59(27%) specimens, leukoplakia and dysplasia of epithelium were present in 21(8%) and 16(6%) specimens, respectively (Fig. 3). Leukoplakia was detected more often in men than in women. This relation was close to reaching statistical significance ($p=0.055$) (Table 2). The presence of dysplastic lesions of the epithelium was correlated with the age of the patients and smoking habit ($p=0.0042$, $p=0.0021$) (Tables 3 and 4). There was no relation between the number of years of smoking and the presence of epithelial dysplasia.

Electron microscopy examination revealed that exudative fluid escaped from subepithelial tissue to the epithelium causing the widening of intercellular spaces. There were a lot of pinocytic vesicles in the peribasal part of the epithelium. In the widened intercellular spaces there were visible protoplasmic processes with intercellular connections of desmosome type. The basement membrane apart from local swelling and breaks revealed normal structure. There were no ribosomes, rough cytoplasmic reticulum and fibrous structures (Fig. 4).

Discussion

Reinke's edema is comparatively frequent cause of voice disorders in the middle age population. Lehman et al. analysed a group of 655 patients with benign laryngeal lesions revealing that Reinke's edema was the third most common disease after polyps and chronic laryngitis [7].

TABLE 2

Relation between sex of the patient and incidence of leukoplakia

Sex	Without leukoplakia	Leukoplakia	p*
Women	190	13	0.055
Men	50	8	
Total	240	21	

p*- chi-square test

TABLE 4

Relation between smoking habit of the patients and dysplastic lesions of laryngeal epithelium

Smoking habit	Lack of dysplasia	Dysplastic lesions	p*
No-smokers	30	1	0.002
Smokers	215	15	
Total	245	16	

p*- chi-square test

There is unresolved problem of histological differentiation between benign pathological changes of the vocal folds such as: Reinke's edema, polyps and vocal nodules. In general, laryngeal polyps have a diverse histological appearance, very often making it impossible to differentiate from Reinke's edema. Therefore close cooperation between a clinician and histopathologist is needed. Detailed description of endoscopic appearance of the lesion may help in avoiding misunderstandings.

In our study there was a high diversity of histological descriptions given by histopathologists in the tissue specimens obtained from patients with clinically diagnosed Reinke's edema although edema was very often noticed in subepithelial region. These observations are in accordance with the results of other authors [2, 12, 15]. Electron microscopic investigations revealed loosened intercellular junctions and widening of intercellular spaces, especially in basal and spinous layers. We found also that a high number of wide microvessels in subepithelial space is a characteristic histologic feature of Reinke's edema. According to Kleinsasser [6] accumulation of liquid in subepithelial spaces is the result of changing vessel permeability. Rak [10] revealed, on the basis of electron microscopy studies, changes in vessels of mucosal lamina propria that confirm the role of microcirculation in the pathogenesis of Reinke's edema.

Interestingly we found that edema of subepithelial tissue is more frequent in women than in men. This observation may be explained by the fact that edema is often connected with sex hormones. Desouza and Ribeiro-Da Silva [5] revealed that the paw edema induced by staphylococcal enterotoxin B (SEB) administration is hormonally regulated. Pillai et al. [9] found that relaxin induced uterine edema and growth is mediated by estrogen receptors [2].

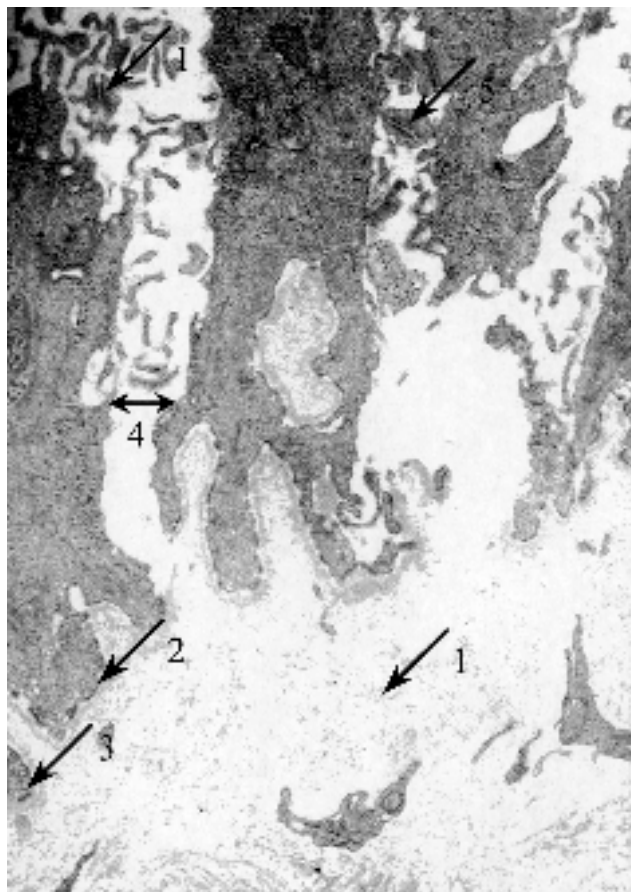


Fig. 4. Transmission electron microscopy. Widening of intercellular spaces (4, 5), a lot of pinocytotic vesicles in the peribasal part of the epithelium (2, 3). In the widened intercellular spaces visible protoplasmic processes with intercellular connections of desmosome type (1). The basement membrane apart from local swelling and breaks revealed normal structure (2). There were no ribosomes, granular cytoplasmic reticulum and fibrous structures.

The determination of incidence of dysplastic lesions of epithelium in patients with Reinke's edema is a very important problem. Although Reinke's edema is a benign lesion, the entire surgically removed mucosa has to be examined histologically to exclude malignancy. Almost all of the patients are heavy smokers so one may expect a comparatively high incidence of precancerous conditions in this group. There are few cases of laryngeal cancer described in patients treated previously for Reinke's edema [3, 14]. Moesgaard Nielsen et al. [8] analysed histopathologic results in 120 patients with Reinke's edema who underwent microsurgery and found mild dysplasia in 30 cases and moderate dysplasia in one case. At the follow-up examination, 19 cases among the patients with dysplastic lesions required a repeated biopsy. All of them had mild dysplasia. One patient from this group, who had been a heavy smoker, developed invasive carcinoma within 13 months. In our study mild and moderate dysplasia were found in 6% of cases. On the basis of literature and our results we may state that Reinke's edema is not a precancer-

ous lesion of the larynx. Nevertheless patients suffering from Reinke's edema, especially those who did not give up smoking, should be regularly examined by the laryngologist.

Conclusions

1. The most characteristic histologic features of Reinke's edema are: edema and a high number of wider microvessels in subepithelial space.
2. Ultrastructurally characteristic features of Reinke's edema are: loosened intercellular junctions and widening intercellular spaces, especially in basal and spinous layers.
3. Reinke's edema is not a precancerous lesion of the larynx.

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