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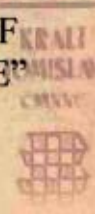
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“FORENSIC APPLICATION OF DNA TECHNOLOGY”



THE LECTURE WILL BE HELD IN MAIN HALL OF
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NORMAL AND PATHOLOGIC STRUCTURE OF VOCAL FOLD REINKE'S SPACE

Santa Večerina-Volić and Tihana Ibrahimpašić

Department of Phoniatics, University Department of ENT, Head and Neck Surgery, Zagreb University Hospital Center, Zagreb, Croatia

SUMMARY – Reinke's space is a very special vocal fold space, which has an important role in phonation. All major vocal fold pathology develop in this space, and a scar or edema in this space is always combined with significant vibratory and acoustic alteration. In spite of this fact, the specificities of Reinke's space structure have not been discovered for years. In the present study, the highly specific Reinke's space structure was demonstrated by use of horizontal serial sections through the vocal fold of human adult larynges. It was found to be composed of parallel connective fibers organized in three layers, each layer characterized by a different ratio in the number of collagenous *versus* elastic fibers. Only a vocal fold with such a regular and specific Reinke's space structure can produce normal human voice.

Key words: *Vocal cords – pathology; Larynx – pathology; Voice disorders – diagnosis; Voice disorders – etiology*

Introduction

The majority of vocal fold pathology develop in the mucosal layer of the vocal fold, precisely in Reinke's space, e.g., vocal nodules, polyps and edema¹ (Fig. 1a, b, c). Modern techniques of vocal fold visualization including show motion mode (demonstrating vocal fold vibrations responsible for production of fundamental laryngeal frequency) have revealed two vibratory elements. The cover (mucosal layer) and the body (vocal muscle) of the vocal fold are vibrating horizontally as a whole, periodically interrupting expiratory air stream and producing fundamental laryngeal tone. Vocal fold mucosa is involved separately in the same act by undulatory mucosal vibrations, so-called mucosal or glottic wave².

If the mucosal or glottic wave is reduced, it is a sign of a scarring process in Reinke's space, or it could be the first manifestation of early glottic cancer (Fig 2). The loss (or reduction) of vibration in one segment or of the whole vocal

fold is a serious sign of a high risk vocal fold lesion and probably malignant alteration³ (Fig 3).

Along with the aforementioned facts, our observations strongly suggested the possibility of a specific vocal fold mucosa, precisely Reinke's space structure. It was B. Reinke^{4,5}, a German anatomist, who was first to describe a very specific space in the vocal fold, i.e. a closed space lined in all directions with firm connective tissue. The space has been named after him as Reinke's space, and the edema involving the region as Reinke's edema. However, Reinke did not notice and describe the specificities of this important space and layer structure of the vocal fold.

The aim of our study was to determine structural specificities of this functionally and pathologically important space.

Material and Methods

Investigating the spread of laryngeal cancer on horizontal sections of human larynx, we noticed a very loose sub-epithelial space of the vocal fold, corresponding to Reinke's space (Fig. 4). Some regularity in its structure was already observed under low magnification. Stimulated by this observation, ten adult larynges (aged 30-50) free from la-

Correspondence to: *Santa Večerina-Volić*, Department of Phoniatics, University Department of ENT, head and neck surgery, Zagreb University Hospital Center, Šalata 2, 10000 Zagreb, Croatia
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ryngeal pathology were selected at Department of Forensic Medicine, Zagreb University School of Medicine. Before the procedure of larynx decalcination, the Reinke's space borders were anatomically revisited. Decalcinated larynges were embedded in celloidine for 8-10 months and then serially cut in horizontal plane (including the base of the epiglottis to the caudal end of the subglottic region). Two hundred 40- to 60- μ m thick sections were obtained from each laryngeal specimen. Only 10 sections corresponding to the middle part of the vocal fold were extracted, so that 100 specimens were histologically analyzed, previously stained with hematoxylin – eosin, Mallory – azan combination, modified Van Gieson and Paf-Halmi. Before histomorphological analysis, the anteroposterior plane of the vocal fold was divided into three portions, anterior, middle and posterior, further subdividing the middle portion into the superficial, intermediate and deep layer. The respective sections of ventricular folds were studied by the same methods for comparison.

Results

Our anatomical investigations confirmed the borders of Reinke's space to correspond to those described by Reinke: *linea arcuata superior* and *inferior* (invagination of the vocal mucosa on the upper *vs.* inferior surface of the vocal fold), medially basilar membrane, laterally vocal ligament, anteriorly connective tissue or anterior commissure, posteriorly insertion of the vocal muscle into the vocal process (Fig. 5). In all glottal specimens (horizontal sections of the vocal fold middle part), a very specific feature was observed: connective fibers were ordered in parallel with the vocal edge and directed in parallel with each other (Fig. 6). The density of the connective tissue varied significantly in the mediolateral direction. High connective fiber density was observed in the superficial layer (just below the epithelium), the intermediate layer was composed of very loose connective tissue, whereas density of the connective fibers ordered in parallel in the deep layer was greatest. We could not delineate this deep layer with vocal ligament. Using Mallory-azan technique, it was impossible to differentiate elastic from collagenous fibers. On the other hand, this technique in particular pointed to an extremely marked structural difference between the ventricular and vocal fold: in the vocal fold, lamina propria fibers were strictly regularly ordered, whereas in the ventricular fold they were completely chaotically distributed. Paf-Halmi and Van Gieson methods enabled visualization and differentiation of elastic *vs.* collagenous fibers. Collagenous

fibers are curved and run in parallel with the vocal fold edge, with only some thin and straight elastic fibers in-between. On the contrary, in the intermediate layer of the vocal fold lamina propria there are only few collagenous fibers and numerous elastic fibers (Fig. 7). The deep layer consists of dense bunches of collagenous fibers exclusively (Fig. 8).

Discussion

Japanese authors have also published a number of reports on their investigation of Reinke's space specificities⁶⁻⁸. They have introduced the term "layer structure of the vocal fold mucosa" and described the layers as the superficial, intermedial and deep one, which in fact represent vocal ligament. They also found a high degree of regular and parallel distribution of connective fibers in Reinke's space. Subotić *et al.*⁹ investigated a series of fetal larynges and found this specific regular structure of the vocal fold mucosa to be present as early as in 15-week-old fetuses.

Based on this knowledge of such a histologic regularity in the vocal fold lamina propria (Reinke's space), we could better understand various phenomena of vibratory and acoustic irregularity found in the mechanism of voice production. In Reinke's edema, the voice is deep, harsh and monotonous. Loose vocal folds are vibrating with a large amplitude and Bernoulli's phenomenon is overexpressed. Bernoulli's phenomenon is a normal component of the normal vibratory process, when the vocal fold mucosa is sucked medially because of negative air pressure. Acoustic and vibratory analyses performed in patients with various types of hoarseness revealed Bernoulli's phenomenon to be absent or only reduced in some patients, or overexpressed in others. Correlation of these data with histologic studies of the tissue obtained on laryngomicroscopic interventions showed Bernoulli's phenomenon to be overexpressed and mucous membrane to be built of scattered, loose connective fibers in Reinke's edema, whereas in reduced (or absent) Bernoulli's phenomenon the mucosa transformed into irregular, dense connective tissue or there was malignant infiltration in some cases¹⁰. The transformation of structural regularity into a chaotic distribution is connected with the high irregularity in vibratory and acoustic parameters. Structural irregularity provokes an irregular glottic wave and alters Bernoulli's phenomenon. Based on this observation, we developed a test of provoked Bernoulli by forced inspiratory phonation: regular and elastic mucosa will move medially, the ballooning phenomenon will be observed in Reinke's edema, whereas in stiff vocal fold (scar or malignant infiltration) no movement will occur.

This fundamental knowledge about structural specificities of Reinke's space was crucial and initiated an enormous number of additional investigations of Reinke's space, including histochemistry, biochemistry, immunohistochemistry and ultrastructural studies.

Nowadays, we are talking about extracellular matrix of Reinke's space^{1,11}, whereby its composition could also be analyzed by various methods including gene expression analysis and, which is most important, correlated with vibratory function, which is by its dynamics of 2000 vibrations *per* second (in soprano) certainly a region of greatest dynamics in human body.

Correlation between the histologic and other tissue specificities with vibratory behavior of the vocal fold helps in getting better insight in both voice physiology and tissue specificities. Vocal folds with their special function and the specific role of Reinke's space in the vibratory process is perhaps the best example of such interaction.

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Sažetak

NORMALNE I PATOLOŠKE STRUKTURE REINKEOVA PROSTORA

S. Večerina-Volić i T. Ibrahimpašić

Reinkeov prostor je specifično građen i omeđen prostor sluznice glasnice koji ima važnu ulogu u fonaciji. Većina glasničke patologije razvija se upravo unutar Reinkeova prostora, a ožiljak ili edem unutar njega uvijek uzrokuje značajne vibracijske i akustične promjene. Unatoč tome, osobitosti strukture Reinkeova prostora su dugo bile nepoznate. Rabeći serijske horizontalne rezove glasnica iz humanih odraslih larinksa pokazana je visoko specifična struktura Reinkeova prostora. Čine ga usporedno poredana vezivna vlakna organizirana u tri sloja. U svakom sloju postoji različit odnos broja kolagenih naspram elastičnih vlakana. Samo glasnice koje posjeduju takvu pravilnu i specifičnu strukturu Reinkeova prostora mogu proizvesti normalan ljudski glas.

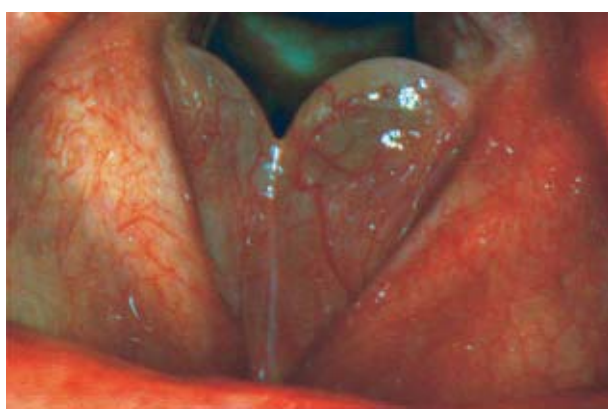
Ključne riječi: *Glasnice – patologija; Larinks – patologija; Poremećaji glasa – dijagnostika; Poremećaji glasa – etiologija*



a



b



c

*Fig. 1. Digital pictures (all pictures are from the Department of Phoniatics archive) of:
(a) vocal fold nodules
(b) a vocal fold polyp
(c) Reinke's edema*



Fig. 2. Digital picture of thin, scarred vocal folds

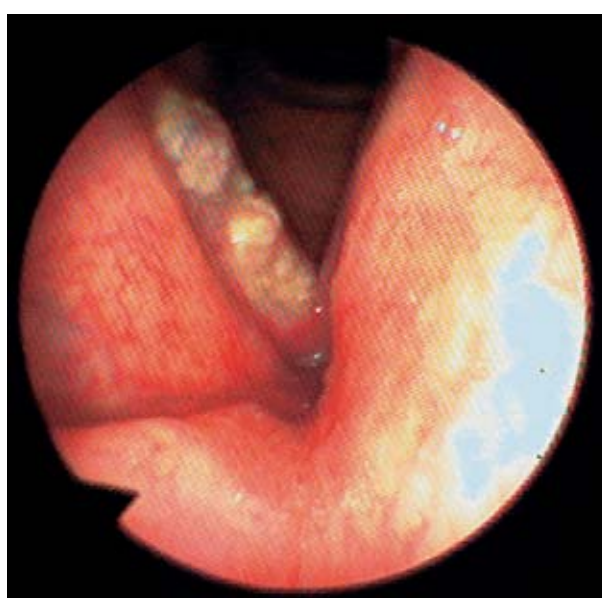


Fig. 3. Digital picture of a vocal fold cancer – complete loss of vibrations

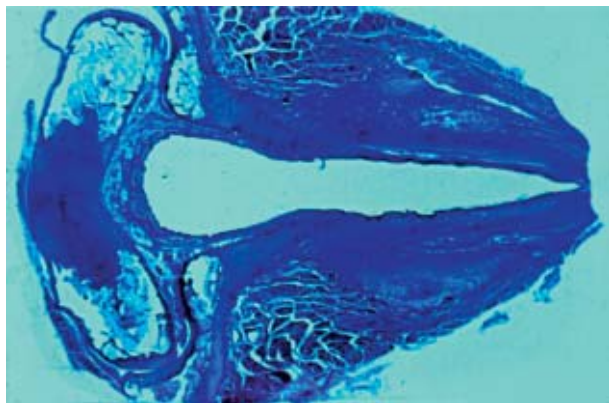


Fig. 4. Horizontal serial sections of human larynx and subepithelial space of a vocal fold.

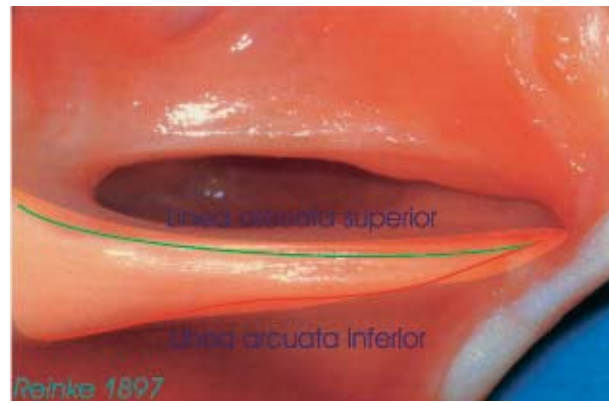


Fig. 5. Reinke's space border (a cadaveric larynx).

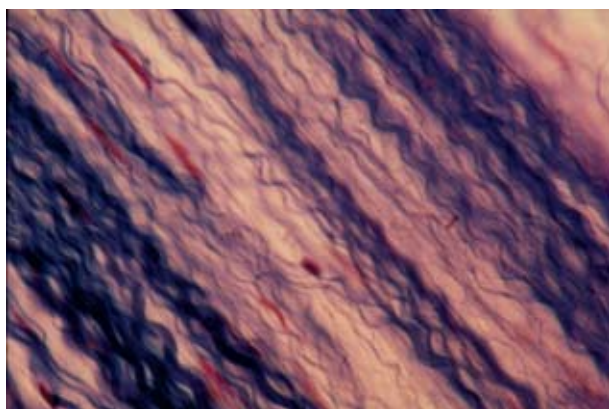


Fig. 6. Greater magnification reveals a higher degree of regularity in the vocal fold lamina propria structure (Mallory – azan staining).

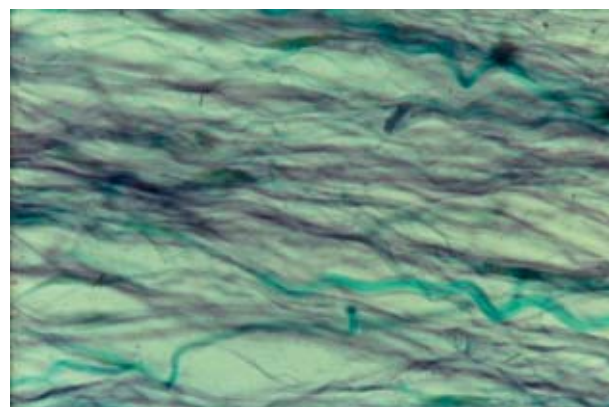


Fig. 7. Intermediate layer of the human vocal fold lamina propria (Palf-Halmi technique).

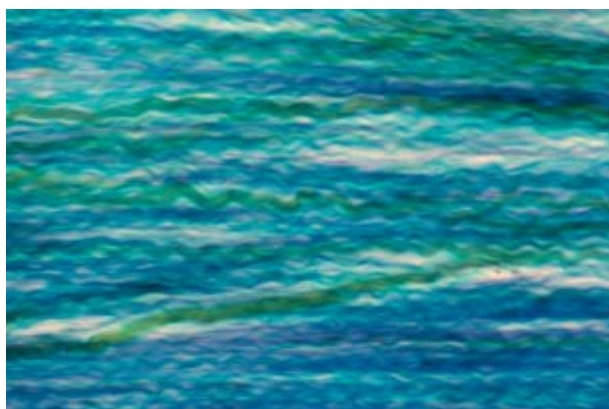


Fig. 8. Deep layer of the human vocal fold lamina propria (Palf-Halmi technique).