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Special guest lecture

Minister of science, education, and sports of Republic of Croatia,
Doc. dr. sc. Dragan Primorac

"Forensic Application of DNA Technology"

The lecture will be held in main hall of Multimedial Center "Sestre Milosrdnice"
University Hospital Zagreb
Friday June 4th, 2004 at 09.15 AM
NORMAL AND PATHOLOGIC STRUCTURE OF VOCAL FOLD REINKE’S SPACE

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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 corneal 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, h, i). 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. Reinke4, a German anatomist, who 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 subepithelial 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-
rygeal pathology were selected at Department of Forensic Medicine, Zagreb University School of Medicine. Before the procedure of larynx decalcification, the Reinke’s space borders were anatomically revisited. Decalcified larynges were embedded in celluloid 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-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 Paff-Halvri. 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 tx. inferior surface of the vocal fold), medially basilar membrane, laterally vocal ligament, anteroinferior 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. Paff-Halvri and Van Gieson methods enabled visualization and differentiation of elastic rx. 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 specificities*4. 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. 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 folds are 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 cases*16. 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 prooved Bernoulli by forced inspiratory phonation: regular and elastic mucosa will move medially, the balloning 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 enor-
mous number of additional investigations of Reinke’s space, including histrochemistry, biochemistry, immunohis-
tochemistry and ultrastructural studies.

Nowadays, we are talking about extracellular matrix of
Reinke’s space\(^1\), whereby its composition could also be
analyzed by various methods including gene expression
analysis and, which is most important, correlated with vi-
bratory function, which is by its dynamics of 2000 vibra-
tions 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 tis-
sue 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.

References

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Sažetak

NORMALNE I PATOLOŠKE STRUKTURE REINKEJOVA PROSTORA

S. VEČERINA VOLJIČ, T. IBRAHIMPASIĆ

Reinkeov prostor je specifično graden in omejen prostor sluznice glasnice ki ima važno ulogo v fonaciji. Večina glasniške
patologije razvija se upravno unutar Reinkeova prostora, a odličaj in tedem unutar njega uvijek izraža znatne vibracijske in
akustične promjene. Unatoč tome, osnovnosti strukturu Reinkeova prostora su druge bile negoiznate. Rabeičverjetnje horizontalne
rezerve glasniške iz humanih odraščenih latinskih pokazana je visoko specifčna struktura Reinkeova prostora, čime ga
usporedno poredana vezivna vlagak organizirani u tri sloja. U svakom sloju postoje različiti odnos broju kolažnih naspram elastičnih
vlakana. Samo glasnice koje posjeduju takvu pravilnu i specifičnu strukturu Reinkeova prostora mogu proizvesti normalan ljudski
glas.

Ključne riječi: Glaznice – patologija; Larnaka – patologija; Porumbošć glas – dijagnostika; Porumbošć glas – etiologija

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Fig. 1. Digital pictures (all pictures are from the Department of Phoniatrics archive) of:
(a) vocal fold nodules
(b) 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 subepi-
pharyngeal 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 (Malbray – azan stain-
ing).

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

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