Are Vocal Alterations Caused by Smoking in Reinke's Edema in Women Entirely Reversible After Microsurgery and Smoking Cessation?

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Summary: Background. Reinke's edema is a benign lesion of the vocal folds that affects chronic smokers, especially women. The voice becomes hoarse and virilized, and the treatment is microsurgery. However, even after surgery and smoking cessation, many patients remain with a deep and hoarse voice.

Objectives. The aim of the present study was to compare pre- and postoperative acoustic and perceptual-auditory vocal analyses of women with Reinke's edema and of women in the control group, who were non-smokers.

Methods. A total of 20 women with videolaryngoscopy diagnosis of Reinke's edema who underwent laryngeal microsurgery were evaluated pre- and postoperatively (6 months) by videolaryngoscopy, acoustic voice, and perceptual-auditory analyses (General degree of dysphonia, Roughness, Breathiness, Asthenia, Strain, and Instability [GRBASI] scale), and the maximum phonation times were calculated. The pre- and postoperative parameters of the women with Reinke's edema were compared with those of the control group of women with no laryngeal lesions, smoking habit, or vocal symptoms.

Results. Acoustic vocal perceptual-auditory analyses and the maximum phonation time of women with Reinke's edema improved significantly in the postoperative evaluations; nevertheless, 6 months after surgery, their voices became worse than the voices of the women from the control group.

Conclusions. Abnormalities caused by smoking in Reinke's edema in women are not fully reversible with surgery and smoking cessation. One explanation would be the presence of possible structural alterations in fibroblasts caused by the toxicity of cigarette components, resulting in the uncontrolled production of fibrous matrix in the *lamina propria*, and preventing complete vocal recovery.

Key Words: Reinke's edema–dysphonia–microsurgery–voice–smoking.

INTRODUCTION

Reinke's edema is a benign lesion of the vocal folds that affects chronic smokers, especially women. The voice becomes hoarse and virilized, explaining why women have more symptoms than men.[1,2] Reinke's edema was classified by Yonekawa[3] into three stages, depending on the extent of the injury, as follows: type 1, superficial edema of vocal folds without obstruction of the glottic lumen; type 2, edema extending to the posterior portion of the vocal folds; type 3, massive edema of the vocal folds that affects its entire length, a small glottic lumen remaining in the posterior region of the larynx.

The treatment of choice in Reinke's edema, especially for types 2 and 3, is microsurgery, which can be performed by various techniques, among them microdebrider, decortication, laser, and microflap.[4–7] Nevertheless, postoperative voice restoration is slow and depends on several factors, especially the success of the surgical removal of the lesion and smoking cessation.

Postoperative voice improvement in Reinke's edema is notorious, especially when compared with that in the preoperative vocal conditions, as recorded by us and many other authors in perceptual-auditory and acoustic voice analyses.[6–8] The voices of some women, however, remain hoarse, despite having abandoned smoking. To prove this statement, the purpose of the present study was to compare the results of the acoustic voice analysis of women with Reinke's edema before and after microsurgery with those of the control group of nonsmoking women.

PATIENTS AND METHODS

The present study included 20 women, aged 40–70 years, with videolaryngoscopy diagnosis of Reinke's edema grades 2 or 3 according to Yonekawa's classification,[3] without symptoms of gastroesophageal reflux or vocal abuse and who had abandoned smoking. All patients were assessed before and after surgery (6 months) by means of videolaryngoscopy using laryngeal telescope (70°, 8 mm, Azap, Baden-Württemberg, Germany) coupled to a multifunctional system (video system type XE-30, Eco X-TFT/USB, Germany) with image recording on DVD; computerized acoustic voice analysis (*Multi-dimensional Voice Program*; KayPentax, Lincoln Park, NJ), perceptual-auditory evaluation (General degree of dysphonia, Roughness, Breathiness, Asthenia, Strain, and Instability [GRBASI] scale), and calculation of the maximum phonation time (MPT).

For acoustic vocal assessments, the vocal samples were obtained during sustained emission of the vowel /a/ and of the phonemes /s/ and /z/. The acoustic parameters analyzed were fundamental frequency (F0—number of cycles/second), jitter percentage (%), shimmer percentage (%), pitch perturbation quotient, amplitude perturbation quotient, soft phonation index, and noise-harmony ratio.

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All vocal evaluations were conducted in a quiet room by the same speech pathologist; a headset microphone (Shure, São Paulo (SP), Brazil) connected to a computer was used to collect the vocal samples. The GRBASI scale was applied in vocal samples of these women previously recorded for the acoustic measures during sustained emission of the vowel /a/ for the perceptual-auditory evaluations. Data were blindly analyzed by three speech therapists with expertise in voice, and there should be agreement on at least two of the scores. The vocal parameters analyzed were G (General degree of dysphonia), R (Roughness), B (Breathiness), A (Asthenia), S (Strain), and I (Instability). The parameters were quantified from 0 to 3, depending on the intensity of voice disorder: 0 (no voice disorder), 1 (mild voice disorder), 2 (moderate voice disorder), and 3 (severe voice disorder).

All women with Reinke's edema in the present study were operated by the same surgeon, using the same surgical technique for microsurgery: incision of laryngeal mucosa parallel to the free edge, aspiration of mucus content, and removal of redundant mucosa, preserving part of the mucosa coverage and the vocal ligament.

The results of the postoperative vocal assessments of women with Reinke's edema were compared with those of the control group, which consists of 40 healthy women, aged from 40 to 70 years, nonsmokers, and without vocal abuse, gastroesophageal reflux, or other comorbidities that could compromise the voice, such as intubation, inhaled irritants, or neck trauma. The participants in the control group underwent the same sequence of vocal evaluation conducted for patients with Reinke's edema.

The project was approved by the Human Research Ethics Committee of the Botucatu Medical School.

Statistical methods

The Wilcoxon test (a nonparametric test used to compare two paired samples) was used to compare the pre- and postoperative values of acoustic parameters, GRBASI scale scores, and MPT. The comparison between the measures of the control group and the postoperative measures of women with Reinke's edema was carried out using Mann-Whitney test (a nonparametric test to compare two independent samples). In all comparisons, the significance level was set at 5%.

RESULTS

Table 1 depicts the pre- and postoperative values of acoustic voice analyses of women with Reinke's edema and of the control group. An important improvement of vocal parameters could be noted when comparing pre- and postoperative values of women with Reinke's edema; however, these values still differ from those of the control group. The F0 values are still lower than those of the voices of the controls, and other acoustic parameters are still above normal ranges. The only acoustic parameter that did not have statistical significance was soft phonation index.

The same behavior could be observed in the vocal analysis of the GRBASI scale; lower scores were recorded in the postoperative period; the postoperative scores nevertheless remained higher than those in the control group, indicating some degree of remaining dysphonia, except for S and I parameters (Table 2). In Table 3, we can see that the values of MPT increased after surgery, but also remained lower than the values in the control group.

DISCUSSION

Reinke's edema is a benign laryngeal lesion directly related to smoking. For some authors, vocal abuse and laryngopharyngeal reflux are also predisposing factors;[1,2] there is no evidence, however, to prove that these factors, when isolated, can develop lesions in their most important presentations, as in stages 2 or 3 of the Yonekawa's classification.[3] Most authors are unanimous in recognizing the direct participation of smoking in

TABLE 1.
Acoustic Vocal Parameters: Average, Standard Deviation, and Statistical Analysis of the Pre- and Postoperative Voices of Patients who Underwent Microsurgery for Reinke's Edema, and Those of the Controls

Moment Acoustic Parameters	Preoperative Reinke's Edema	Postoperative Reinke's Edema	Control	*P* Value
F0	149.8 ± 33.8	182.6 ± 34.1	220.5 ± 28.3	Pre × Post < 0.001* Post × Control < 0.001*
Jitter%	2.9 ± 1.9	1.6 ± 0.8	0.71 ± 0.42	Pre × Post < 0.001* Post × Control < 0.001*
PPQ	1.8 ± 1.3	0.9 ± 0.5	0.40 ± 0.23	Pre × Post –< 0.001* Post × Control < 0.001*
Shimmer%	9.3 ± 7.7	5.0 ± 2.1	2.20 ± 1.1	Pre × Post < 0.001* Post × Control < 0.001*
APQ	6.5 ± 5.5	3.6 ± 1.5	1.6 ± 0.7	Pre × Post < 0.019* Post × Control < 0.001*
NHR	0.236 ± 0.158	0.147 ± 0.041	0.117 ± 0.019	Pre × Post < 0.001* Post × Control < 0.004*
SPI	12.0 ± 7.0	11.6 ± 9.9	9.4 ± 4.5	Pre × Post = 0.866 Post × Control = 0.946

* Statistical significance.
Abbreviations: APQ, amplitude perturbation quotient; F0, fundamental frequency; NHR, noise-harmony ratio; PPQ, pitch perturbation quotient; SPI, soft phonation index.

TABLE 2.
Median (Minimum and Maximum) and Statistical Analysis of GRBASI Scores of the Voices of Patients Before and After Microsurgery for Reinke's Edema, and Those of the Controls

Moment GRBASI Scale	Preoperative Reinke's Edema	Postoperative Reinke's Edema	Control	*P* Value
G	2 (1–3)	1 (0–2)	0.5 (0–1)	Pre × Post < 0.01* Post × Control < 0.01*
R	2 (1–3)	1 (0–2)	0 (0–1)	Pre × Post < 0.01* Post × Control < 0.01*
B	1 (0–2)	1 (0–2)	0 (0–1)	Pre × Post = 0.129 Post × Control < 0.01*
A	0 (0–0)	0 (0–0)	0 (0–0)	
S	1 (0–2)	0 (0–1)	0 (0–1)	Pre × Post < 0.01* Post × Control < 0.01*
I	1 (0–3)	0 (0–2)	0 (0–1)	Pre × Post < 0.01* Post × Control = .035*

* Statistical significance.
Abbreviation: GRBASI, General degree of dysphonia, Roughness, Breathiness, Asthenia, Strain, and Instability.

Reinke's edema, reported by almost 100% of patients in many studies.[1,2,9,10] In a recent survey from our service, analyzing the causes of dysphonia in 2019 patients, Reinke's edema was responsible for 9.2% of the cases, who are all smokers and two out of three are women.[11]

The vocal alterations caused by Reinke's edema are gradual and evolve with significant reduction in the fundamental frequency of the voice, which is the main incentive to perform surgery, because the injury is not spontaneously reabsorbed. The surgical procedure may be conducted by several techniques, all following the same principle of removing the mucus content deposited in Reinke's space, and excising the redundant mucosa, with maximum preservation of the structure of the vocal folds, including epithelium, *lamina propria*, and vocal ligament.[4–6,12] Recently, Young et al[13] described the benefits of the titanyl phosphate laser to the surgery of the Reinke's edema. The authors reviewed nine patients submitted to potassium-titanyl-phosphate laser treatment for Reinke's edema and observed decrease of voice handicap index (VHI; 10 by 8.23) without other complications. An important benefit of this surgical technique is the nonablative treatment or ablation without tissue removal. So, titanyl phosphate laser can be safely and effectively used in Reinke's edema.[13]

Considerable voice improvement is observed after surgery for Reinke's edema, as published by us and by other authors.[4–8] However, there is evidence that surgery cannot eliminate all structural alterations present in the vocal folds, justifying the remaining symptoms and the vocal characteristics of those with Reinke's edema, which differ from those of the voices of control patients, nonsmokers, as evidenced in this study. One explanation could be the persistence of structural changes in the *lamina propria* caused by Reinke's edema; understanding them further is key for the treatment of these abnormalities.

Histological studies on Reinke's edema have found, in more than 80% of the cases, epithelial hyperplasia, thickening of the basement membrane, inflammation, and fibrosis.[10,14–17] Pastuszek et al[10] have added findings of electron microscopy, especially the loss of epithelial intercellular junctions occupied by amorphous material, indicating severe edema of the epithelium.

An increase in the number of blood vessels of the *lamina propria* is also noted in Reinke's edema.[16,17] Probably, nicotine components participate in the regulation of angiogenesis,

TABLE 3.
Averages of the Maximum Phonation Time (MPT) for the Vowel /a/ and the Phonemes /s/ and /z/ of the Voices of Patients Submitted to Microsurgery for Reinke's Edema, Comparing Pre- and Postoperative Results With Those of the Controls

Moment MPT	Preoperative	Postoperative	Control	*P* Value
/a/	7.3 ± 2.9	8.6 ± 2.7	14.6 ± 4.3	Pre × Post = 0.092 Post × Control < 0.01*
/s/	9.2 ± 3.9	9.7 ± 5.4	13.2 ± 5.0	Pre × Post = 0.612 Post × Control < 0.01*
/z/	8.1 ± 3.8	9.3 ± 3.6	14.1 ± 3.8	Pre × Post = 0.179 Post × Control < 0.01*

* Statistical significance.
Abbreviation: MPT, maximum phonation time.

resulting in increased vascular permeability, and therefore the formation of subepithelial edema.[18]

Sakae et al[19] performed histological analysis with picrosirius red in 20 vocal folds with Reinke's edema and observed complete derangement of collagen fibers in the midst of myxoid stroma, especially in the deeper layers of the *lamina propria*. These findings allow us to assume that the fibroblasts participate in the pathophysiology of this process because they are responsible for the production of most components of the extracellular matrix and of the fibrous matrix. In Reinke's edema, the production of collagen fibers would be altered as a result of toxic chemical mediators found in cigarettes. Días-Flores et al,[20] in a study on immunohistochemistry, observed an increase in density and morphological abnormalities in the population of fibroblasts in the *lamina propria* of Reinke's edema. These cells are arranged in a myxoid stroma, along with macrophages, and present positive immunoexpression for vimentin, CD34, and CD10, markers for hematopoietic cells, and, to a lesser extent, CD99, marker of mesenchymal tumors. Martins et al[21] demonstrated positive immunostaining for laminin, collagen IV, and fibronectin in Reinke's edema, which participate in the repair process of injured tissues.

According to Thibeault,[22] the behavior of Reinke's space when faced with aggressors is unclear. There seems to be alterations in gene expression of the components of the extracellular matrix, controlled by the fibroblasts, resulting in failures in remodeling the *lamina propria*.

As we have seen, although Reinke's edema is a very common laryngeal lesion, its pathophysiology is complex and deserves further studies, in particular at the molecular level for better understanding.

CONCLUSIONS

The abnormalities caused by smoking in women with Reinke's edema are not fully reversible with surgery and smoking cessation. One explanation would be the presence of possible structural alterations in fibroblasts caused by the toxicity of cigarette components, resulting in the uncontrolled production of fibrous matrix in the *lamina propria*, and preventing complete vocal recovery.

REFERENCES

1. Pickhard A, Reiter R. Benign vocal fold lesions. *Laryngorhinootologie*. 2013;92:304–312.
2. Hah JH, Sim S, An SY, et al. Evaluation of the prevalence of and factors associated with laryngeal diseases among the general population. *Laryngoscope*. 2015;125:2536–2542.
3. Yonekawa H. A clinical study of Reinke's edema. *Auris Nasus Larynx*. 1988;15:57–78.
4. Burduk PK, Wierzchowska M, Orzechowska M, et al. Assessment of voice quality after carbon dioxide laser and microdebrider surgery for Reinke edema. *J Voice*. 2015;29:256–259.
5. Stanković P, Vasić M, Djukić V, et al. Vocal fold masses removal–the sub epithelial micro flap technique. *Acta Chir Iugosl*. 2008;55:43–47.
6. Szkiełkowska A, Miaśkiewicz B, Włodarczyk E, et al. Assessment of voice in patients with Reinke's oedemas treated with laser microsurgery. *Otolaryngol Pol*. 2008;62:82–87.
7. Dursun G, Ozgursoy OB, Kemal O, et al. One-year follow-up results of combined use of CO2 laser and cold instrumentation or Reinke's edema surgery in professional voice users. *Eur Arch Otorhinolaryngol*. 2007;264:1027–1032.
8. Sommer C, Schultz-Coulon HJ. Long-term results after microlaryngoscopic removal of Reinke's edema. *HNO*. 2007;55:365–374.
9. Garcia Alvarez CD, Campos Banales ME, Lopes Campos D, et al. Polyps, nodules and Reinke edema. An epidemiological and histopathological study. *Acta Otolaryngol*. 1999;50:443–447.
10. Pastuszek P, Krecicki T, Zalesska-Krecicka M, et al. Histological and electron microscopic investigation of Reinke's edema. *Pol J Pathol*. 2003;54:61–64.
11. Martins RH, do Amaral HA, Tavares EL, et al. Voice disorders: etiology and diagnosis. *J Voice*. 2015;doi:10.1016/j.jvoice.2015.09.017; In press.
12. Bouchayer M, Cornut G. Microsurgical treatment of benign vocal fold lesions: indications, technique, results. *Folia Phoniatr (Basel)*. 1992;44:155–184.
13. Young VN, Mallur PS, Wong AW, et al. Analysis of potassium titanyl phosphate laser settings and voice outcomes in the treatment of Reinke's edema. *Ann Otol Rhinol Laryngol*. 2015;124:216–220.
14. Dikkers FG, Nikkels PG. Benign lesions of the vocal folds: histopathology and phonotrauma. *Ann Otol Rhinol Laryngol*. 1995;104(9 pt 1):698–703.
15. Remacle M, Lagneau E, Doylen A, et al. Exudative laryngeal diseases of Reinke's space. *Ann Otolaryngol Chir Cervicofac*. 1995;109:33–38.
16. Dikkers FG, Nikkels PGJ. Lamina propria of the mucosa of benign lesions of the vocal folds. *Laryngoscope*. 1999;109:1684–1689.
17. Volic SV, Kaplan I, Seiwerth S, et al. Extracellular matrix of Reinke's space in some pathological conditions. *Acta Otolaryngol*. 2004;124:505–508.
18. Berchtold CM, Coughlin A, Kasper Z, et al. Paracrine potential of fibroblasts exposed to cigarette smoke extract with vascular growth factor induction. *Laryngoscope*. 2013;123:2228–2236.
19. Sakae FA, Imamura R, Sennes LU, et al. Disarrangement of collagen fibers in Reinke's edema. *Laryngoscope*. 2008;118:1500–1503.
20. Díaz-Flores L, Gutiérrez R, del Pino García M, et al. CD34-positive fibroblasts in Reinke's edema. *Laryngoscope*. 2014;124:E73–E80.
21. Martins RH, Domingues MA, Fabro AT, et al. Reinke's edema: immunoexpression study of fibronectin, laminin and collagen IV in 60 cases by immunohistochemical techniques. *Braz J Otorhinolaryngol*. 2009;75:821–825.
22. Thibeault S. Advances in on understanding of the Reinke space. *Curr Opin Otolaryngol Head Neck Surg*. 2005;13:147–151.