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Histological changes of rat soft palate with exposure to experimental laryngopharyngeal reflux

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Abstract

Objective: The possible effects of laryngopharyngeal reflux (LPR) on laryngeal and otologic disorders have been studied in the literature. There have been no reports explaining the possible effects of LPR on the soft palate. Therefore, in this study, we investigated the histopathologic changes in the rat soft palate using an experimental model of reflux.
Subjects and methods: Eighteen healthy 200–220-g 20-week-old Sprague-Dawley rats were used. The animals were divided into three groups according to exposure time (1, 4, and 12 week exposures), and four rats were examined as controls who had undergone sham operation. An experimental model of gastroesophageal reflux was induced under general anesthesia. After exposure, the animals were sacrificed, and their soft palates were removed. The histopathological changes in the soft palates were observed under a light microscope.
Results: Submucous gland hyperplasia, inflamation, subepithelial edema, vascular engorgement, muscular atrophy and dilated glandular excretory duct were compared among the groups. Submucous gland hyperplasia, subepithelial edema, inflammation, vascular engorgement, muscular atrophy and dilated glandular excretory duct were significantly different in the exposure groups compared with the control group.
Conclusion: On the basis of histopathological evaluations, our findings suggest that reflux affects the soft palate, which suggests that these pathological changes may reflect the relationship between LPR and airway obstruction.

Keywords: Reflux; Soft palate; Histopathology

1. Introduction

The diagnosis of laryngopharyngeal reflux (LPR) has become more prevalent than in past years. Laryngopharyngeal reflux (LPR) has been suggested as a term for the association of laryngeal disorders and gastroesophageal reflux (GER) [1]. It is estimated that 4–10% of patients presenting to ear, nose, and throat (ENT) physicians have a diagnosis of LPR [2,3]. Laryngopharyngeal reflux (LPR) is an important etiologic factor in the development of many disorders of the upper aerodigestive tract. The most common clinical presentations of LPR include hoarseness, chronic cough, throat clearing, and globus sensation. Dysphagia has also been reported [4].

In the literature, the possible effects of LPR on nasal, laryngeal and otologic disorders have been studied. But studies focusing on soft palate, which is a part of the upper aerodigestive tract, have not been encountered.

Most common studies of soft palate were related to obstructive sleep apnea, primary snoring and velopharyngeal incompetence [5–7]. Recently, the histopathologic changes of soft palate in obstructive sleep apnea (OSA) were also studied [8].

Some studies have investigated the relationship between OSA and GER, although several have reported some pathophysiological mechanisms, there is not any study focusing on the relationship between OSA and histological changes of soft palate with exposure to gastric acid reflux.

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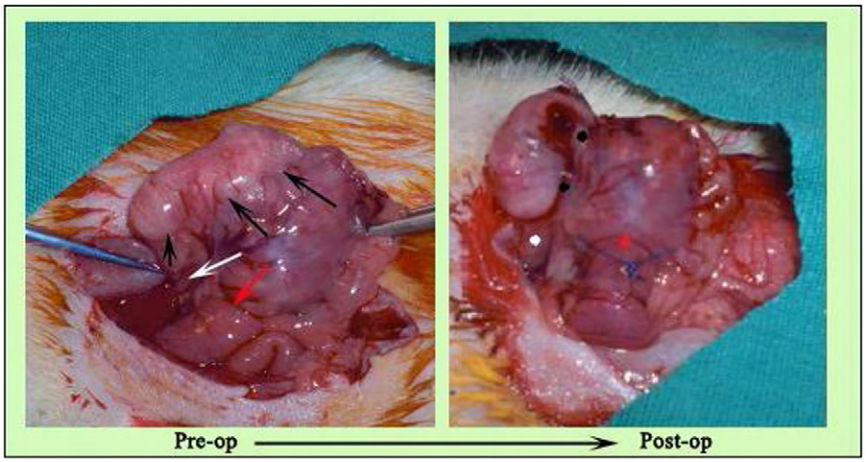

Fig. 1. The experimental surgical technique of LPR: ligated limiting ridge is marked with black points, the esophagus is marked with white point; and the involvement of the pyloric stenosis by covering the duodenum near the pyloric ring with a piece of catheter is marked with a red point.

Therefore, in this study, we investigated the histopathologic changes in the rat soft palate using an experimental model of reflux, and compared these data with the histopathological changes of soft palate in OSA.

2. Subjects and methods

2.1. Study design and setting

All of the animals were treated in accordance with protocols approved by the Institutional Animal Care and Use Committee. Eighteen healthy 200–220-g 20-week-old Sprague-Dawley rats were used. The animals were divided into three groups according to exposure time (1, 4, and 12 week exposures), and four rats were examined as controls who had undergone sham operation.

2.2. Surgical procedure

We used the surgical procedure to achieve experimental LPR which have found acceptance in the literature before. Preoperatively, the animals were fasted for 12 h. Intraperitoneal anesthesia was administered with a 1:1 mixture of 20 mg/mL xylazine chloride and 100 mg/mL ketamine chloride, using 0.1 mL of the solution for each 100 g weight. After removing the hair from the abdomen, the skin was cleaned with 10% polyvinylpyrrolidone iodine. A 2-cm midline laparotomy incision was made, starting at the xiphoid process, and the peritoneal cavity was inspected. LPR was induced using the upper abdominal midline pyloric stenosis plus limiting ridge ligation method [9]. The pyloric stenosis involved covering the duodenum near the pyloric ring with a small piece of an 18 Fr Nelaton catheter while the transitional region between the forestomach and the glandular portion (limiting ridge) was ligated using a non-absorbable suture (Fig. 1). Sham-operated rats, receiving only a midline incision, served as a control group. After the surgical procedure, the animals were allowed to recover from anesthesia in individual cages and were then allowed water early on the first postoperative day. Beginning the second postoperative day, the rats were fed a regular daily diet. The animals were observed daily under the supervision of the veterinarian in charge. The rats in the experimental model of LPR groups were sacrificed after exposure for 1, 4, or 12 week and compared with the control group. All rats were sacrificed by injecting a lethal dose of pentobarbital sodium; they were subsequently decapitated and their soft palates were removed.

2.3. Postoperative evaluation of LPR

Before the sacrification of rats, to evaluate GER and presence of reflux at the oropharynx, barium contrast (E-Z-HD

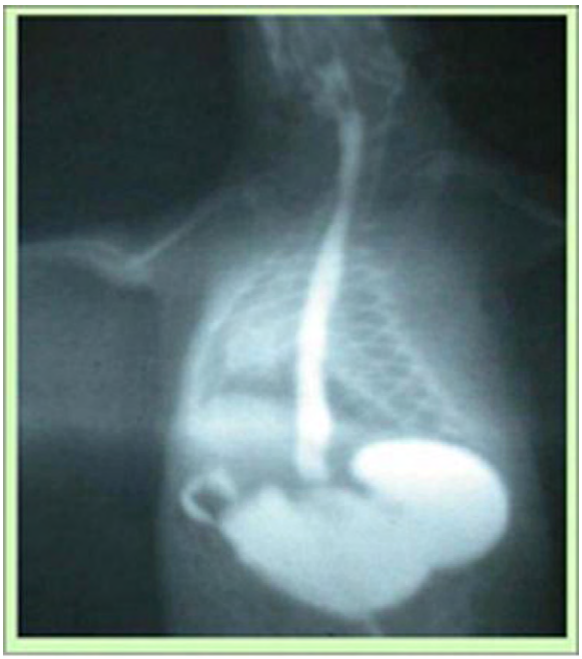

Fig. 2. Evaluation of postoperative laryngopharyngeal reflux.

Table 1
Comparison of changes in soft palate.

	Submucous gland hypertrophy	Subepithelial edema	Inflammation	Vascular engorgement	Muscular atrophy	Ductus dilatationu
Cont-Gr.1	0.127	0.040*	0.040*	0.008**	0.046*	1.00
Cont-Gr.2	0.036*	0.026*	0.025*	0.007**	0.009**	0.005**
Cont-Gr.3	0.023*	0.009**	0.020*	0.009**	0.007*	0.009**
Gr.1-Gr2	0.074	0.176	0.264	0.371	0.411	0.005**
Gr.1-Gr.3	0.030*	0.007**	0.322	0.074	0.100	0.009**
Gr.2-Gr.3	0.212	0.042*	0.050*	0.221	0.221	0.134

Mann–Whitney *U*-test was used to evaluate post hoc subgroup analysis.
* $P < 0.05$.
** $P < 0.01$.

barium sulfate) was administered via an 18 Fr Nelaton catheter. Radiographs were used to determine the presence or absence of barium at the oropharynx (Fig. 2).

2.4. Pathologic evaluation

All 18 specimens consisted of the distal portion of the soft palate. The soft palates were immediately fixed in 10% buffered formalin, processed in the usual manner, embedded in paraffin blocks, and serially cut in the sagittal plane. Deparaffinized 4-μm thick midsagittal sections were mounted on glass slides and stained with hematoxylin and eosin (H&E) to study the morphmetric and qualitative histopathologic features of the sections. Light microscopy was used at a magnification of 40, 100 and 400 to compare the findings among the four groups of the study. All sections were coded to avoid observers' bias during examination. The histopathological changes were considered as:

2.4.1. Submucous gland hyperplasia

At a magnification of 100, a score was assigned according to the number of submucous glands visible in a section: 0 (less than three glands); 1 (3–10 glands); 2 (11–30 glands); or 3 (30 glands).

2.4.2. Inflamation

At a magnification of 400, the number of lymphocytes present in the submucosa was scored as follows: 0 (20 lymphocytes); 1 (21–50 lymphocytes); 2 (51–80 lymphocytes); 3 (81–120 lymphocytes); 4 (120 lymphocytes); and/or at a magnification of 400, the number of PMN cell present in submucosa was scored as follows: 0 (none); 1 (one or two PMNs); 2 (three to 10 PMNs); or 3 (10 PMNs).

2.4.3. Subepithelial edema, vascular engorgement, muscular atrophy and dilated glandular excretory duct

At a magnification of 100, a score was assigned according to the degree of subepithelial edema, vascular engorgement and dilated excretory duct: 0 (none); 1 (mild); 2 (moderate); or 3 (marked).

2.5. Statistical analysis

All statistical calculations were performed with NCSS 2007&PASS 2008 Statistical Software (Utah, USA). Besides standard descriptive statistical calculations (mean, median and standard deviation), Kruscal–Wallis test was used in the assessment of parameters according to groups, Mann–Whitney *U*-test was used for the evaluation of differences. The statistical significance level was established at $P < 0.05$ and confidence interval were 95%.

3. Result

3.1. Submucous gland hyperplasia

When we assessed submucous gland hyperplasia between the control group and the study groups, there were significant difference ($P < 0.05$). The detailed comparisons of the differences between the groups are as follows: The comparison of submucous gland hyperplasia between control group and 1 week group revealed no significant difference ($P = 0.127$). However, the comparison of submucous gland hyperplasia between control group and 4 week and 12 week groups revealed significant difference, respectively ($P = 0.036$, $P = 0.023$). When we compared 1 week group and 4 week group, there was no significant difference ($P = 0.074$). On the other hand, there was significant difference between the 1 week group and 12 week group ($P = 0.030$). There was no significant difference between 4 week and 12 week group ($P = 0.212$), (Table 1), (Figs. 3 and 4a and b).

3.2. Subepithelial edema

When we assessed subepithelial edema between the control group and the study groups, there were significant difference ($P < 0.05$). The detailed comparison of the differences between the groups are as follows: The comparison of subepithelial edema between the control group and 1 week, 4 week, and 12 week groups revealed significant difference, respectively ($P = 0.040$, 0.026,

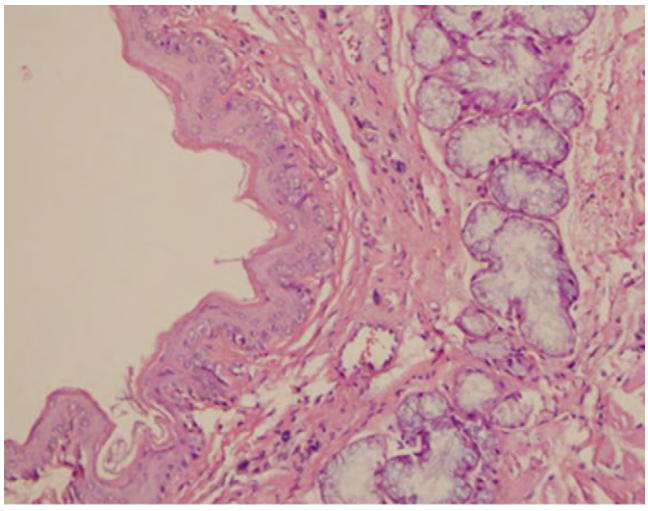

Fig. 3. Normal soft palate mucosa in sham-operated rats (HE&E 200×).

0.009). When we compared 1 week group and 4 week group, there was no significant difference ($P = 0.176$). On the other hand, there was significant difference between 1 week group and 12 week group ($P = 0.007$). Also there was significant difference between 4 week and 12 week group, either ($P = 0.042$), (Table 1), (Figs. 3 and 5a and b).

3.3. Inflammation

When we assessed inflammatory cell infiltration between the control group and the study groups, there were significant difference ($P < 0.05$).

The comparison of inflammation between the control group and 1 week, 4 week, and 12 week groups revealed significant differences, respectively ($P = 0.040$, 0.025, 0.020). When we compared 1 week group and 4 week, 12 week groups, there were no significant difference, respectively ($P = 0.264$, $P = 0.322$). On the other hand, there was significant difference between the 4 week group and 12 week group ($P = 0.050$), (Table 1), (Figs. 3 and 6a and b).

3.4. Vascular engorgement

The comparison of vascular engorgement between the control group and the study groups revealed significant difference ($P < 0.05$). The detailed comparison of the differences between the groups are as follows: The comparison of vascular engorgement between the control group and 1 week, 4 week, 12 week groups revealed significant differences, respectively ($P = 0.008$, 0.007, 0.009). But when we compared 1 week group and 4 week, 12 week groups, there were no significant difference, respectively ($P = 0.371$, $P = 0.074$). Also there was no significant difference between 4 week and 12 week group ($P = 0.221$), (Table 1).

3.5. Muscular atrophy

The assessment of muscular atrophy between the control group and the study groups revealed significant difference ($P < 0.05$). The detailed comparison of the differences between the groups are as follows: the comparison of muscular atrophy between the control group and 1 week, 4 week, 12 week groups revealed significant difference, respectively ($P = 0.046$, 0.009, 0.007). But when we compared 1 week group and 4 week, 12 week groups, there were no significant differences, respectively ($P = 0.411$,

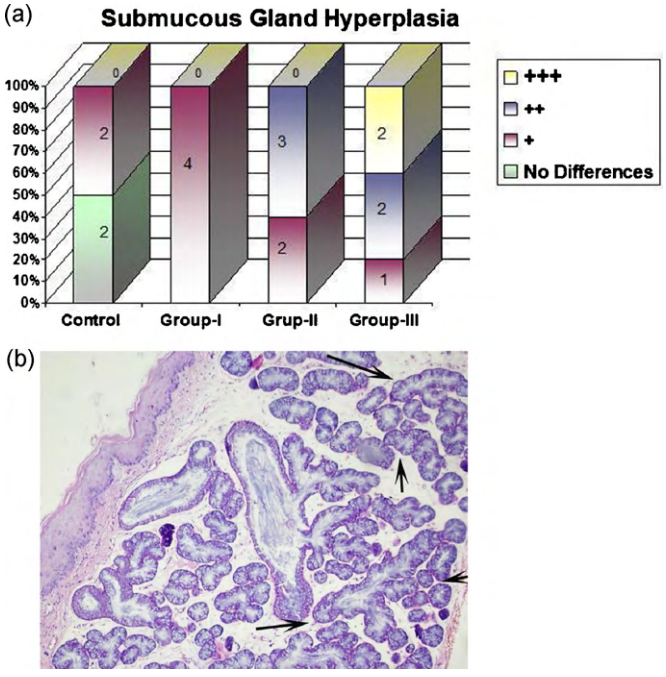

Fig. 4. (a) Submucous gland hyperplasia distribution of the groups. (b) Submucous gland hyperplasia in soft palate mucosa (HE&E 100×).

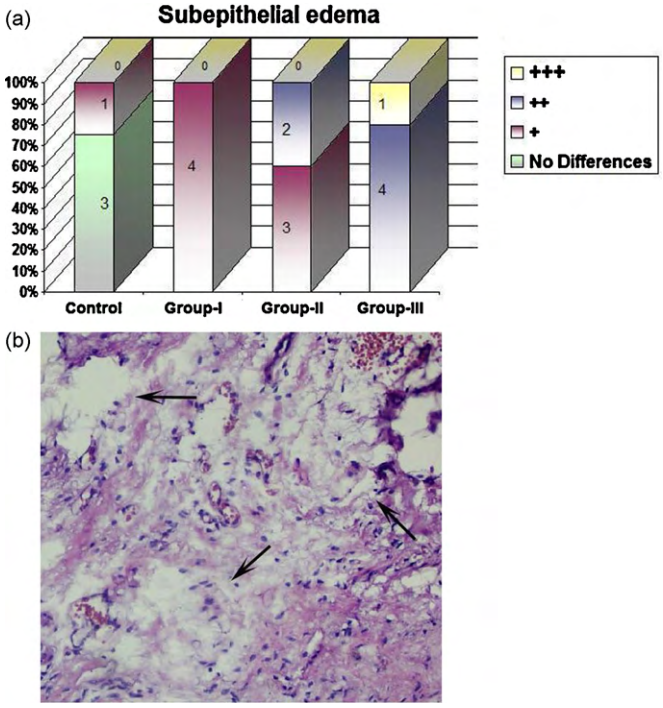

Fig. 5. (a) Subepithelial edema distribution of the groups. (b) Moderate subepithelial edema in soft palate mucosa (HE&E 200×).

$P = 0.100$). Also there was no significant difference between 4 week and 12 week group ($P = 0.221$), (Table 1).

3.6. Dilated glandular excretory duct

When we assessed dilated glandular excretory duct between the control group and the study groups, there were significant difference ($P < 0.05$). The detailed comparisons of the differences between the groups are as follows: the comparison of dilated glandular excretory duct between the control group and the 1 week group revealed no significant difference ($P = 1.00$). However, the comparison of dilated glandular excretory duct between the control group and 4 week, 12 week groups

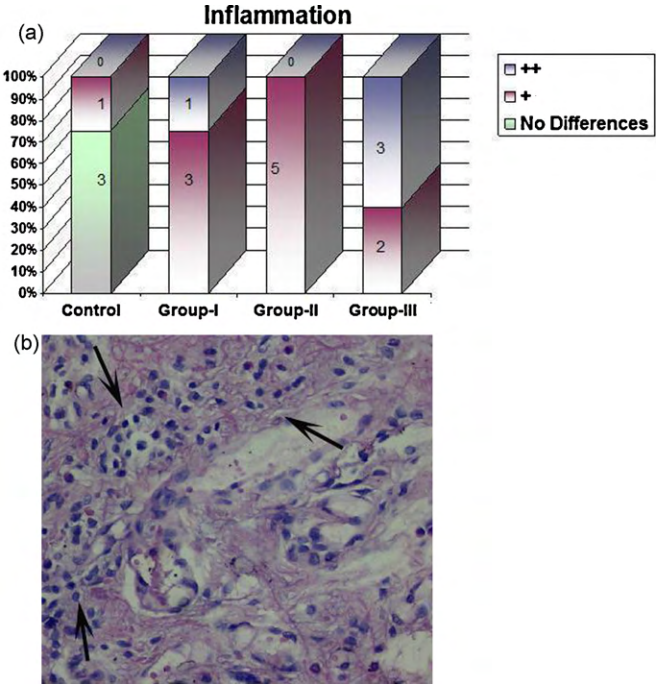

Fig. 6. (a) Inflammation distribution of the groups. (b) +++ Inflammation in soft palate mucosa (HE&E 400×).

revealed significant differences, respectively ($P = 0.005$, $P = 0.009$).

When we compared 1 week group and 4 week, 12 week groups, there were significant differences, respectively ($P = 0.005$, $P = 0.009$). On the other hand, there was no significant difference between 4 week group and 12 week group ($P = 0.134$), (Table 1).

4. Discussion

Gastroesophageal reflux disease (GERD) causes several symptoms in many people, and hence otolaryngologists encounter patients with GERD. In supraesophageal lesions, GERD can affect manifestations in the larynx, pharynx, nasal cavity and middle ear [10]. Although the association of GER with ENT symptoms has already been reported, there have been no reports explaining the relationship between the objective histological findings of the upper aerodigestive tract with exposure to acid reflux and these manifestations.

Recently, as accepted animal models of LPR were described in the literature [9,11,12], the histopathological investigations have been reported. These animal studies have revealed that an acidic pH and the presence of pepsin can cause laryngeal complications and Eustachian tube dysfunction as a result of LPR [13]. Yazici et al. [14] found that nasopharyngeal exposure to experimental reflux altered the Eustachian tube mucosa histopathology and its relation to otitis media.

Soft palate which is a part of upper aerodigestive tract may be affected with exposure to acid reflux. Several histopathologic studies were performed that used qualitative, quantitative, or morphmetric measures to assess the composition and the pathologic features of the soft palate and uvula of patients with OSA [15–17]. Also, according to some studies, GER has been suggested to have an association with OSA [18]. Although these studies have investigated the relationship between OSA and GER, these relationships have not been clearly explained yet.

We hypothesized that reflux mediates histopathologic changes in the soft palate and causes soft palate dysfunction. Therefore, the aim of the present investigation was to evaluate histopathological changes of rat soft palate with exposure to chronic gastric acid reflux and we suggested a new perspective to the relationship between LPR and OSA.

Woodson et al. [15] found, in the soft palate of obstructed patients, hypertrophy of mucus glands, edema of the lamina propria, atrophy of palatal muscles, and demyelinization of peripheral nerve fibers. Others found a significant rise in muscle and fat tissue in the uvula of these patients [16,19], whereas Hamans et al. [17] have recently reported a significant reduction in muscle and a nonsignificant difference in fat tissue between apneic and nonapneic snorers and control subjects [8]. In our study, we found submucous gland hyperplasia, edema of the lamina propria and muscular atrophy.

Inflammation was characterized by edema and mononuclear cell infiltration in the mucosa of the uvula [20]. Woodson et al. [15] supported these observations and found extensive edema of the lamina propria with vascular dilation in the distal soft palate and uvula of snorers and patients with OSA. Consequently, it was postulated that the inflammatory process increased the thickness of the velum and narrowed the upper airway. These changes induce increased resistance during sleep that eventually leads to increased intraluminal negative pressure and upper airway collapse. Berger et al. [8] found inflammatory cell infiltration in OSA patients. Nevertheless, the role of inflamation in the soft palate of patients with OSA is uncertain as yet. The work of Stauffer et al. [19] made no mention of this pathology. Also, in our study we found significant edema and inflamation.

Berger et al. [8] found that vascular engorgement and dilated glandular ducts were observed in a portion of patients with OSA and control subjects, implying that these pathologic changes probably reflect airway obstruction. Here, significant vascular engorgement and dilated glandular ducts were observed in this study.

5. Conclusion

Our findings suggest that reflux affects the soft palate, and increase in the exposure time also increases these histopathologic changes. The present data introduces significant changes in histopathologic data of the soft palate, which were also seen in OSA, when submucous gland hyperplasia and dilatation, subepithelial edema, inflamation, fibrosis, muscular atrophy and dilated glandular excretory duct were considered, implying that these pathological changes may reflect the relationship between LPR and the sequela of airway obstruction.

References

[1] Knight RE, Wells JR, Parrish RS. Esophageal dysmotility as an important co-factor in extraesophageal manifestations of gastroesophageal reflux. Laryngoscope 2000;110(September (9)):1462–6.
[2] Katz PO. State of the art: extraesophageal manifestations of gastroesophageal reflux disease. Rev Gastroenterol Disord 2005;5(Summer (3)):126–34.
[3] Vaezi MF. Extraesophageal manifestations of gastroesophageal reflux disease. Clin Cornerstone 2003;5(4):32–8 [discussion 39–40].
[4] Klinkenberg-Knol EC. Otolaryngologic manifestations of gastro-oesophageal reflux disease. Scand J Gastroenterol 1998;33(Suppl. 225): 24–8.
[5] Klotz DA, Howard J, Hengerer AS, Slupchynskj O. Lipoinjection augmentation of the soft palate for velopharyngeal stress incompetence. Laryngoscope 2001;111(December (12)):2157–61.
[6] Haraldsson PO, Karling J, Lysdahl M, Svanborg E. Voice quality after radiofrequency volumetric tissue reduction of the soft palate in habitual snorers. Laryngoscope 2002;112(July (7 Pt 1)):1260–3.

[7] Fischer Y, Khan M, Mann WJ. Multilevel temperature-controlled radiofrequency therapy of soft palate, base of tongue, and tonsils in adults with obstructive sleep apnea. Laryngoscope 2003;113(October (10)):1786–91.
[8] Berger G, Gilbey P, Hammel I, Ophir D. Histopathology of the uvula and the soft palate in patients with mild, moderate, and severe obstructive sleep apnea. Laryngoscope 2002;112(February (2)): 357–63.
[9] Gaia Filho EV, Goldenberg A, Costa HO. Experimental model of gastroesophageal reflux in rats. Acta Cir Bras 2005;20(November–December (6)):437–44 [Epub 2005 Nov 8].
[10] Weaver EM. Association between gastroesophageal reflux and sinusitis, otitis media, and laryngeal malignancy: a systematic review of the evidence. Am J Med 2003;115(August (Suppl. 3A)):81S–9S [Review].
[11] White DR, Heavner SB, Hardy SM, Prazma J. Gastroesophageal reflux and eustachian tube dysfunction in an animal model. Laryngoscope 2002;112(June (6)):955–61.
[12] Tugtepe H, Tugay M, Bozkurt S, Yildiz F, Utkan T, Yegen BC, et al. Esophageal smooth muscle reactivity is impaired in chronic reflux esophagitis by both receptor- and nonreceptor-mediated mechanisms. J Pediatr Surg 2007;42(April (4)):641–6.
[13] Gaynor EB. Gastroesophageal reflux as an etiologic factor in laryngeal complications of intubation. Laryngoscope 1988;98(September (9)): 972–9.
[14] Yazici ZM, Sari M, Uneri C, Midi A, Tugtepe H. Histologic changes in eustachian tube mucosa of rats after exposure to gastric reflux. Laryngoscope 2008;118(May (5)):849–53.
[15] Woodson BT, Garancis JC, Toohill RJ. Histopathologic changes in snoring and obstructive sleep apnea syndrome. Laryngoscope 1991;101(December (12 Pt 1)):1318–22.
[16] Zohar Y, Sabo R, Strauss M, Schwartz A, Gal R, Oksenberg A. Oropharyngeal fatty infiltration in obstructive sleep apnea patients: a histologic study. Ann Otol Rhinol Laryngol 1998;107(February (2)):170–4.
[17] Hamans EP, Van Marck EA, De Backer WA, Creten W, Van de Heyning PH. Morphometric analysis of the uvula in patients with sleep-related breathing disorders. Eur Arch Otorhinolaryngol 2000;257(4):232–6.
[18] Gonzalez ER, Castell DO. Respiratory complications of gastroesophageal reflux. Am Fam Physician 1988;37(February (2)):169–72.
[19] Stauffer JL, Buick MK, Bixler EO, Sharkey FE, Abt AB, Manders EK, et al. Morphology of the uvula in obstructive sleep apnea. Am Rev Respir Dis 1989;140(September (3)):724–8.
[20] Sekosan M, Zakkar M, Wenig BL, Olopade CO, Rubinstein I. Inflammation in the uvula mucosa of patients with obstructive sleep apnea. Laryngoscope 1996;106(August (8)):1018–20.