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How does reflux affect laryngeal tissue quality? An experimental and histopathologic animal study

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ABSTRACT

OBJECTIVE: To investigate the histopathologic changes in intact laryngeal epithelium and mucosa exposed to endogenous gastric acid and pepsin in an experimental model of reflux.

STUDY DESIGN: Randomized trial.

SETTING: The study was conducted at the animal care facility of Haydarpasa Numune Education and Research Hospital.

SUBJECTS AND METHODS: Eighteen healthy 200- to 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 underwent sham operation. An experimental model of gastroesophageal reflux was induced. After exposure, the animals were euthanized, and their larynges were removed. The histopathologic changes in the larynx were observed under a light microscope.

RESULTS: The mean scores for inflammation in the control, one-, four-, and 12-week groups were 0.75 ± 0.50, 1.75 ± 0.50, 2.20 ± 0.45, and 1.94 ± 0.87, respectively. However, mean scores for vascular engorgement in the control, one-, and four-week groups were 0, and in the 12-week group was 2.0 ± 0.70. The mean scores for subepithelial edema in the control, one-, four-, and 12-week groups were 1.00 ± 0, 1.75 ± 0.95, 1.80 ± 0.45, and 2.20 ± 0.84, respectively. However, mean scores for keratinization for the control, one-, and four-week groups were 0, and for the 12-week group was 1.60 ± 0.55. When we compared inflammation, vascular engorgement, subepithelial edema, and keratinization mean scores between the control and study groups, there were statistically significant increases ($P = 0.005$, $P = 0.001$, $P = 0.043$, $P = 0.002$, respectively).

CONCLUSION: Our findings suggest that reflux induces significant histopathologic changes in larynx mucosa.

Reflux of the gastric contents through the upper esophageal sphincter without retching and vomiting is known as extra-esophageal reflux, and once the refluxate enters the pharynx, it is known as laryngopharyngeal reflux.[1] Because of the larynx's anatomic proximity to the upper esophageal sphincter, it is the most important respiratory-tract organ affected by acid reflux. As a result of reflux, the deleterious effects of acid and active pepsin on mucosa appear to be associated with various laryngological problems.[2]

Unlike the stomach, the larynx does not have intrinsic resistance mechanisms such as bicarbonate production in response to gastric acid, a mucosal barrier, or peristalsis. Therefore, although epithelial destruction starts when pH levels are less than four in the esophagus, it starts even when pH levels are greater than five in the larynx.[2] Therefore, laryngopharyngeal reflux (LPR) is believed to be the cause of many laryngeal disorders, such as laryngitis, globus pharyngeus, dysphonia, dysphagia, chronic throat clearing, laryngeal contact ulcers, laryngomalacia, posterior laryngitis, laryngeal granulomas, stridor, and subglottic stenosis.[1,3]

Most investigators agree that the incidence of LPR is increased in patients with laryngeal disorders;[4] however, there are few studies concerning the possible histopathologic effects on laryngeal mucosa exposed to acid reflux due to LPR. Several studies used exogenous acid and pepsin mixtures to apply laryngeal mucosa via canules.[5,6] This study, however, explores the histopathologic changes of intact laryngeal epithelium and mucosa exposed to endogenous gastric acid and pepsin in an experimental model.

Subjects and Methods

Study Design and Setting

All of the animals were treated in accordance with protocols approved by the Marmara University Animal Care and Use Ethical Committee. The study was conducted at the animal care facility of Haydarpasa Numune Education and Research Hospital. Eighteen healthy, 200- to 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-

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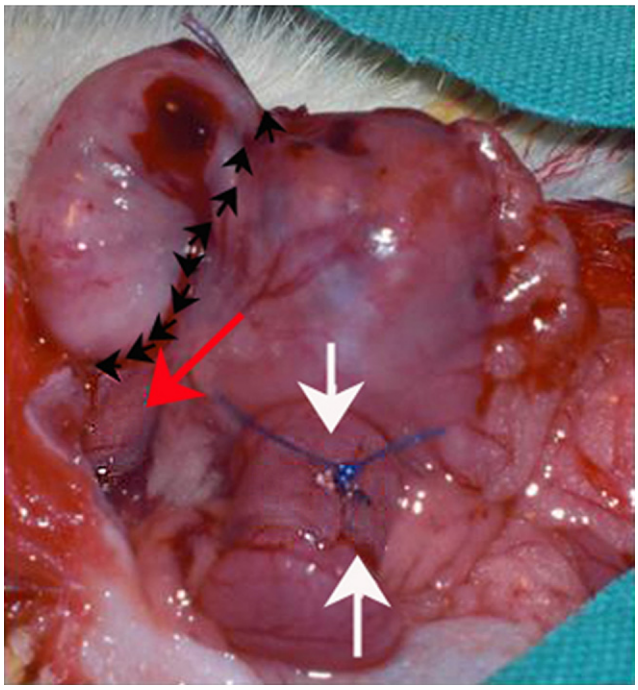

Figure 1 Experimental surgical procedure to induce GER. Pyloric stenosis marked by *white arrows*, limited ridge ligation marked by *black arrows*, and esophagus marked by *red arrow*.

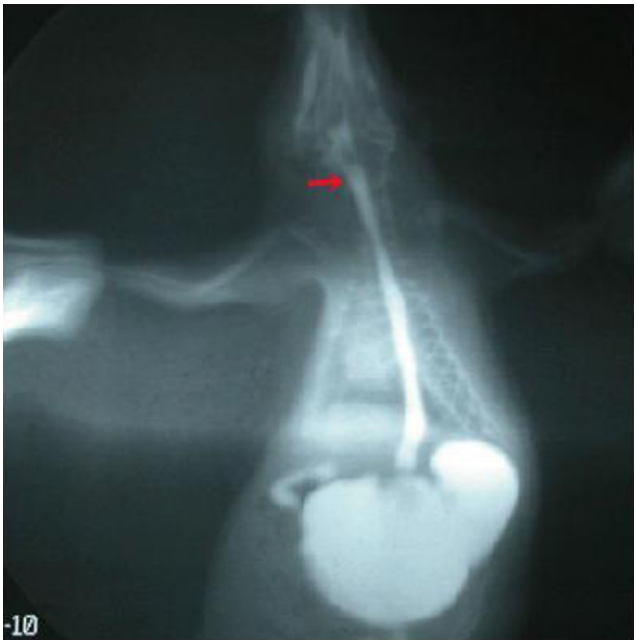

Figure 2 Postoperative evaluation of GER by using radiography with barium contrast. The *red arrow* indicates presence of barium contrast in the oropharynx and larynx, which is evidence of laryngopharyngeal reflux.

week exposures), and four rats that underwent sham operation were examined as controls.

Surgical Procedure

Preoperatively, the animals were fasted for 12 hours. 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 of weight. After removing the hair from the abdomen, the skin was cleaned with 10 percent polyvinylpyrrolidone iodine. A 2-cm midline laparotomy incision was made, starting at the xiphoid process, and the peritoneal cavity was inspected. Gastroesophageal reflux (GER) was induced using the upper abdominal midline pyloric stenosis and limiting ridge ligation method.[7] The pyloric stenosis involved covering the duodenum near the pyloric ring with a small piece of an 18F Nelaton catheter (Qingdao, China) while the transitional region between the forestomach and the glandular portion (limiting ridge) was ligated using a nonabsorbable 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 GER groups were euthanized after exposure for one, four, or 12 weeks and compared with the control group. All rats were euthanized by injection of a lethal dose of pentobarbital sodium; they were subsequently decapitated, and their larynges were removed.

Postoperative Evaluation of GER

Before euthanizing the rats, barium contrast (E-Z-HD barium sulfate) was administered via an 18F Nelaton catheter to evaluate GER and the presence of reflux at the oropharynx. Radiographs were used to determine the presence or absence of barium at the oropharynx (Fig 2).

Pathologic Evaluation

All 18 specimens consisted of the larynx. The larynges were immediately fixed in 10 percent 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 to study the morphometric 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. All sections were coded to avoid observer bias during examination. The histopathologic changes examined were inflammation in the lamina propria and epithelium, and subepithelial edema, vascular engorgement, and keratinization.

Inflammation in the lamina propria and epithelium. 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