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# Dental and oropharyngeal lesions in rats with chronic acid reflux esophagitis

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ABSTRACT

*Objective:* In this study, we evaluated pathological changes in the tooth and pharynx of GERD rats to elucidate the association between gastric acid reflux and oral and pharyngeal diseases.
*Methods:* An experimental rat model of chronic acid reflux esophagitis was surgically created. The oral cavities were observed histologically every 2 weeks until 20 weeks after surgery.
*Results:* At 10 weeks after surgery, molar crown heights in GERD rats were shorter than that in control rats, and inflammatory cell infiltration by gastric acid reflux was found in the periodontal mucosa of GERD rats. Furthermore, dental erosion progressed in GERD rats at 20 weeks after surgery, and enamel erosion and dentin exposure were observed. During the same period, inflammatory cell infiltration was observed in the mucosa of the posterior part of the tongue. These findings suggest that gastric acid reflux may be one of the exacerbating factors of dental erosion, periodontitis and glossitis.
*Conclusion:* We investigated oral changes in an experimental rat model of GERD and observed development of dental erosion, periodontitis and glossitis. Our findings suggested chronic gastric acid reflux may be involved in the pathogenesis of oral disease.


## 1. Introduction

The number of patients with gastroesophageal reflux disease (GERD) is increasing annually due to changes in diet, increases in older populations and advances in diagnostic technique with the use of endoscopy [1]. Various otolaryngological diseases, such as globus pharyngeus, laryngeal granuloma and chronic cough, are thought to be extra-esophageal manifestations of GERD and are widely recognized as laryngopharyngeal reflux diseases (LPRD). To understand the mechanism and pathogenesis of LPRD, we surgically created a rat model of GERD to observe histological changes in the pharynx, larynx and lower airways caused by gastric acid reflux in previous studies. Inflammatory changes were observed in the arytenoid mucosa within 20 weeks after surgery [2]. Moreover, laryngeal granuloma developed due to mechanical injury [3]. In the lower airways, chronic pneumonia and progression of inflammatory cell infiltration around the vagal nerve in the thoracic esophagus were observed at 16 weeks after surgery, indicating that gastric acid reflux is associated with the pathogenesis of chronic cough and aspiration pneumonia [2]. In addition, we reported pathological changes observed in GERD rats at 50 weeks after surgery were similar to pathological findings of human pulmonary fibrosis [4]. In the present study, we observed pathological changes in the oral cavity and pharynx using the same experimental animal model of GERD to elucidate the association between gastric acid reflux and oral and pharyngeal diseases.

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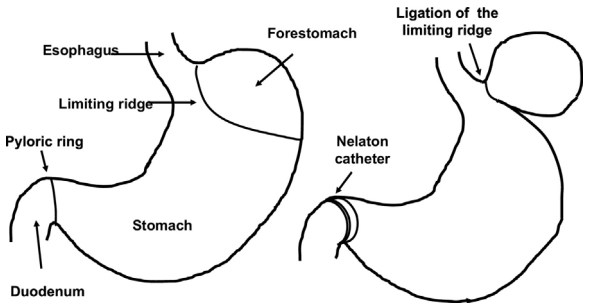


**Fig. 1.** Preparation of the GERD rat model.
After laparotomy, a strip of 18-Fr Nelaton catheter was tied around the pyloric sphincter. The transitional region between the forestomach and the glandular portion (limiting ridge) was ligated with 2-0 silk thread.

**Table 1**
Crown height of the first molar.

| | mean ± SE (mm) |
|---|---|
| Control group at 10 weeks after surgery (n = 2) | 0.84 ± 0.02 |
| GERD rats at 10 weeks after surgery (n = 6) | 0.65 ± 0.04 |
| Control group at 20 weeks after surgery (n = 2) | 0.76 ± 0.01 |
| GERD rats at 20 weeks after surgery (n = 6) | 0.64 ± 0.02 |

Statistically significant differences were observed between the GERD rats and the control group at both 10 and 20 weeks after surgery ($p < 0.05$ by Student's t-test).

## 2. Materials and methods

All aspects of these experiments were conducted according to the guidelines provided by the Ethical Committee of Experimental Animal Care at Saga University. Male Wister rats (8 weeks old, 200–250 g) were purchased from KBT Oriental Co. Ltd (Saga, Japan). All animals were fed standard chow under standard laboratory conditions (room temperature 22 ± 2 °C, relative humidity 55 ± 5% and a 12-h light-dark cycle). They were allowed to acclimate to this environment for 1 week prior to surgery. The GERD rat model was prepared as previously described by Omura et al. [5]. Rats fasted overnight and were anesthetized by inhalation anesthesia using ether. The abdomen was opened via a 3-cm-long, midline incision and a piece of 18-Fr Nelaton catheter was placed around the area of the pyloric sphincter to restrict gastric emptying. The transitional region between the forestomach and the glandular portion (limiting ridge) was ligated with 2-0 silk thread (Fig. 1). Rats fasted for 48 h after surgery but were allowed to drink water. Sham-operated rats, which received only a midline incision, served as the control group.

We prepared 30 GERD rats and 5 control rats. The pharynx, larynx and oral cavity were excised under inhalation anesthesia using ether. In the GERD group, three rats were sacrificed every 2 weeks from 10 weeks until 20 weeks after surgery; one rat was also sacrificed every 2 weeks from 10 weeks until 20 weeks after surgery in the control group. We measured the distance from the gingival border to the mesial and distal cusp tips on the lingual side, and calculated the mean value of these measurement as the crown height of the first molar of the mandible. We used Student's t-test to compare crown heights between the GERD rats and the control group. $P < 0.05$ was considered statistically significant. Tissue samples were collected and fixed in 10% buffered formalin for 24 h. Formalin-fixed tissue samples were then embedded in paraffin blocks to maintain tissue orientation for histopathological analysis. Tissue sections (5 μm) were mounted onto glass slides and stained with hematoxylin and eosin.

## 3. Results

Ten out of the 30 GERD rats died by 10 weeks after surgery (survival rate: 67%). The major causes of death were perforation and hemorrhage of the digestive tract. Two GERD rats died between 10 and 20 weeks as a result of malnutrition and ileus. Three GERD rats remained alive at 20 weeks after surgery. These rats were sacrificed at 22 weeks after surgery, but no obvious pathological changes were observed in the molars and oropharynx in comparison with the rats sacrificed at 20 weeks (data not shown). In the control group, all rats lived to 20 weeks after surgery. The mean body weight of GERD rats was decreased compared with that of control rats at 10 weeks after surgery (280 g vs 360 g, respectively).

Regarding oral changes, crown height of the first molars at 10 and 20 weeks after surgery, were significantly shorter in the GERD rats than in the control rats (Table 1). In the pathological findings, molar crown heights in the GERD rats (Fig. 3A) were shorter than those in the control rats (Fig. 2A). Additionally, inflammatory cell infiltration was observed in the periodontal

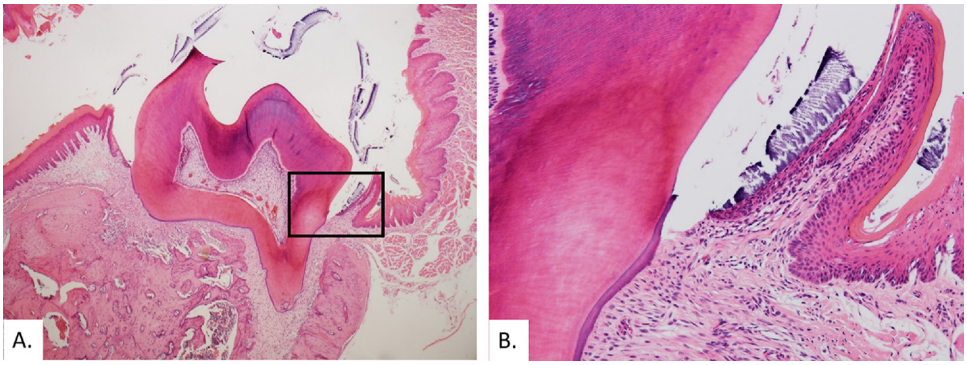


**Fig. 2.** Representative image of tooth and periodontal tissue from a rat in the control group.
A. Representative image of a tooth from a rat in the control group (original magnification, 2×).
B. Representative image of periodontal tissue from a rat in the control group (original magnification, 10×).

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