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Pepsin-induced Differential IL-6 Expression in Laryngeal and Laryngopharyngeal Tissue: Non-invasive Rat Model for Laryngopharyngeal Reflux

*Adelien Adelien [1], Irfannuddin Irfannuddin[2], Hardi Darmawan[2], Agus Surono[3], Krisna Murti[4], Debby Handayati Harahap[5], Elvie Zulka Kautzia Rachmawati[6]

Abstract

Introduction:

Laryngopharyngeal reflux is a common condition caused by exposure of the upper aerodigestive tract to gastroduodenal content, resulting in inflammation to the area. Despite the condition being well documented in the human population, the literature on animal models for laryngopharyngeal reflux is currently limited. The aim of this study is to create a non-invasive model of laryngopharyngeal reflux using rats to investigate changes in IL-6 expression in the laryngeal and laryngopharyngeal regions.

Materials and Methods:

Male Wistar rats, ranging from ages 10-14 weeks, were exposed to 0.5% bovine pepsin dissolved in artificial gastric juice three times daily for 5-15 days via a modified IV cannula in several volumes (0 mL, 0.1 mL, 0.15 mL, and 0.2 mL) according to the treatment groups. Subsequently, laryngeal and laryngopharyngeal tissue IL-6 concentrations were measured through ELISA on allotted days of sacrifice.

Results:

Significant differences in laryngeal and laryngopharyngeal tissue IL-6 concentration were measured ($p = 0.005$ and $p = 0.020$ respectively). Laryngeal expression of IL-6 was related to the volume administered ($p = 0.007$) but not to the duration of exposure ($p = 0.338$), with no significant interaction between duration of exposure and administered volume ($p = 0.515$). On the other hand, laryngopharyngeal expression of IL-6 was related to the duration of exposure ($p< 0.001$) but not to the volume administered ($p = 0.521$) with significant interaction between duration and volume ($p < 0.001$).

Conclusion:

The study established the feasibility of using a non-invasive rat model for studying laryngopharyngeal reflux. Additionally, the study demonstrated the differential responses of IL-6 to the addition of the acid-pepsin insult, with dose-dependent IL-6 responding more in laryngeal tissue and time-dependent IL-6 responding in laryngopharyngeal tissue.

Keywords: Animal model, laryngopharyngeal reflux, Tissue IL-6

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[1]*Biomedical Science Doctoral Program, Faculty of Medicine, Sriwijaya University, Indonesia.*
[2]*Physiology Department, Faculty of Medicine, Sriwijaya University, Indonesia.*
[3]*Otorhinolaryngology Department, Faculty of Medicine and Public Health, Gadjah Mada University, Indonesia.*
[4]*Anatomical Pathology Department, Faculty of Medicine, Sriwijaya University, Indonesia.*
[5]*Pharmacology Department, Faculty of Medicine, Sriwijaya University, Indonesia.*
[6]*Otorhinolaryngology Department, Faculty of Medicine, Universitas Indonesia, Indonesia.*
***Corresponding author:** *E-mail: adelien@unsri.ac.id*

Introduction

Laryngopharyngeal reflux is a condition marked by upper aerodigestive tract damage due to exposure to gastroduodenal refluxate. Clinically, laryngopharyngeal reflux is commonly marked by hypersecretion of mucus and inflammation-associated mucosal damage (1,2).

Laryngopharyngeal reflux is also called silent reflux (i.e. not associated with heartburn or regurgitation) (1), although patients may complain of globus sensation, hoarseness, increased mucosal secretion, postnasal drip, laryngitis and pharyngitis, laryngeal erythema, and diffuse laryngeal edema (3,4).

Laryngopharyngeal reflux is also known to cause DNA damage because of chronic exposure to activated pepsin (5).

Nevertheless, the current animal model of laryngopharyngeal reflux is sorely lacking. Rat models used by Shimazu et al. were created by invasive laparotomy and subsequent placement of pyloric ligation (6).

In this study, we aimed to create a non-invasive rat model of laryngopharyngeal reflux and elaborate on changes in IL-6 expression in the laryngeal and laryngopharyngeal regions.

Materials and Methods

Male Wistar rats, with ages ranging from 10-14 weeks and weights ranging from 175-250 grams, were exposed to either 0.5% bovine pepsin (HIMEDIA®No. RM10942) dissolved in sterile artificial gastric juice (Solarbio No. A7921) with pH of 1.5 or sterile physiological saline as control. For the purposes of this study, the IV cannula was modified through removal of the needle and cutting of the cannula 2 cm from the proximal end to allow direct application of pepsin-gastric juice mixture or physiological saline in the posterior pharyngeal region.

The pepsin-gastric juice mixture (given at volumes of 0.1 mL, 0.15 mL, and 0.2 mL) and physiological saline (0.1 mL) were applied three times a day for 5-15 days through the aforementioned modified IV cannula with the aid of a 1 mL syringe.

The animals were housed in communal cages of appropriate size, adjusted to a standard 12-hour light/dark cycle with temperatures ranging between 20-25ºC. All animals were housed at the Research Animal Laboratory, Faculty of Medicine, Sriwijaya University. The grouping of animals is shown below in Table 1.

Table 1. Animal grouping

Days	Volume			
	0.1 mL normal saline (n = 5/group)	**0.1 mL pepsin-gastric juice (n = 5/group)**	**0.15 mL pepsin-gastric juice (n = 5/group)**	**0.2 mL pepsin-gastric juice (n = 5/group)**
5	A	D	G	J
10	B	E	H	K
15	C	F	I	L

On each of the allotted days, the animals from each application group were sacrificed and dissected. The laryngeal and laryngopharyngeal tissue was removed for analysis by tissue IL-6 ELISA (Elk Biotechnology ELK1158) in accordance with the instructions provided in the kit. The ELISA was performed at the Biomedical Laboratory, Faculty of Medicine, Sriwijaya University. Subsequent data analyses were conducted through Stata 16.1SE and Microsoft Excel. These analyses were conducted through one-way ANOVA and two-way ANOVA with interaction terms as appropriate. All values have been presented as a mean ± standard deviation range along with the level of statistical significance. This study has been approved by our Institutional Review Board (Certificate No. 036-2025).

Results

Animal weight ranged from 191.2 ± 42.01 grams (group L) to 282.2 ± 10.03 grams (group E). No significant weight differences were observed, shown in Table 2 below.

Table 2. Baseline body weight

Group	Body weight (gram)	*p*-value
A	258.2 ± 35.57	0.052
B	245.4 ± 44.61	
C	225.0 ± 22.21	
D	255.8 ± 36.26	
E	282.8 ± 10.03	
F	239.8 ± 14.48	
G	257.6 ± 43.42	
H	255.8 ± 42.51	
I	229.8 ± 25.24	
J	260.4 ± 51.89	
K	252.8 ± 43.34	
L	191.2 ± 42.01	

Laryngeal IL-6 concentration ranged from 203.48 ± 83.87 ng/gram (group C) all the way to 468.93 ± 183.33 ng/gram (group J). On average, the highest laryngeal IL-6 concentrations were observed in the groups belonging to day 5 (groups D, G, J) and the lowest laryngeal IL-6 concentrations were observed in the groups belonging to day 10 (groups E, H, K). Statistically significant differences (p=0.005) were observed between groups. All relevant data is presented in Table 3 below.

Table 3. Laryngeal IL-6 concentration

Group	Laryngeal IL-6 (ng/gram)	*p*-value
A	277.51 ± 142.45	0.005
B	211.89 ± 108.57	
C	203.48 ± 83.87	
D	454.16 ± 177.55	
E	234.03 ± 111.08	
F	259.50 ± 85.85	
G	478.96 ± 95.82	
H	262.93 ± 116.88	
I	357.71 ± 195.82	
J	468.93 ± 183.33	
K	319.62 ± 119.03	
L	246.34 ± 116.27	

The highest laryngeal IL-6 tissue concentrations occurred on day 5 and day 10 when 0.2 mL of pepsin-gastric juice was applied. However, the relationship flipped on day 15, where IL-6 concentration was lowest in group L (0.2 mL) instead, as shown in Figure 1 below.

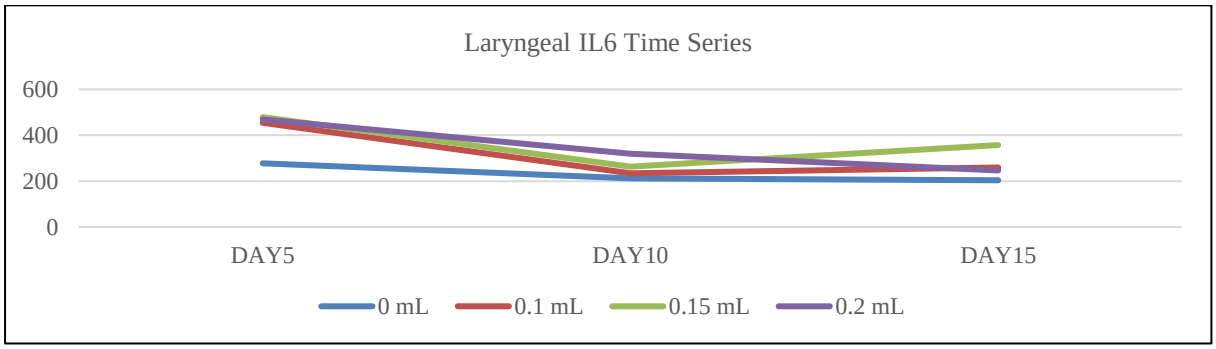

Figure1. Laryngeal IL-6 concentration as time series

We found there was a significant effect of pepsin-gastric juice volume on laryngeal IL-6 concentration (p = 0.007), whereas day of exposure did not have a significant effect on laryngeal IL-6 concentration (p = 0.338). A detailed presentation of the results of the statistical analysis can be seen in Table 4.

Table 4. Laryngeal IL-6 concentration as effect of volume and day

Variable	Partial SS	df	F	*p-value*	*p-value*
Volume	134361.38	1	7.83	0.007	
Day	38021.78	2	1.11	0.338	
Day#Volume	23052.186	2	0.67	0.515	< 0.001
Residuals	926955.07	54			

Laryngopharyngeal IL-6 concentration ranged from 159.52 ± 117.59 ng/gram (group A) all the way to 916.23 ± 664.11 ng/gram (group C). In the treatment group, the lowest laryngopharyngeal IL-6 concentration was observed in the 5-day, 0.1 mL pepsin-gastric group (group D), while the highest laryngopharyngeal concentration of 485.29 ± 178.38 ng/gram was observed in the 15-day, 0.1 mL pepsin-gastric group (group F).

On average, the highest laryngeal IL-6 concentrations were observed in the groups belonging to the 15-day cohort (groups F, I, L), and the lowest laryngeal IL-6 concentrations were observed in the groups belonging to the 5-day cohort (groups D, G, J).

Significant differences (p = 0.020) were observed between groups. The laryngopharyngeal IL-6 concentration is presented in Table 5 below.

Table 5. Laryngopharyngeal IL-6 concentration

Group	Laryngopharyngeal IL-6 (ng/gram)	*p*-value
A	159.52 ± 117.59	
B	297.31 ± 191.98	
C	916.23 ± 664.11	
D	222.12 ± 116.26	
E	293.86 ± 235.25	
F	485.29 ± 178.38	0.020
G	362.08 ± 225.50	
H	394.35 ± 57.68	
I	399.01 ± 172.63	
J	441.33 ± 356.42	
K	423.72 ± 268.73	
L	325.67 ± 181.88	

Regarding time series data, the change in laryngopharyngeal IL-6 levels possessed a time-dependent association. While the 0.1 mL and 0.15 mL pepsin-gastric juice groups showed approximately linear increase, the 0.2 mL pepsin-gastric juice group showed approximately linear decrease of IL-6 with respect to time, as shown in Figure 2 below.

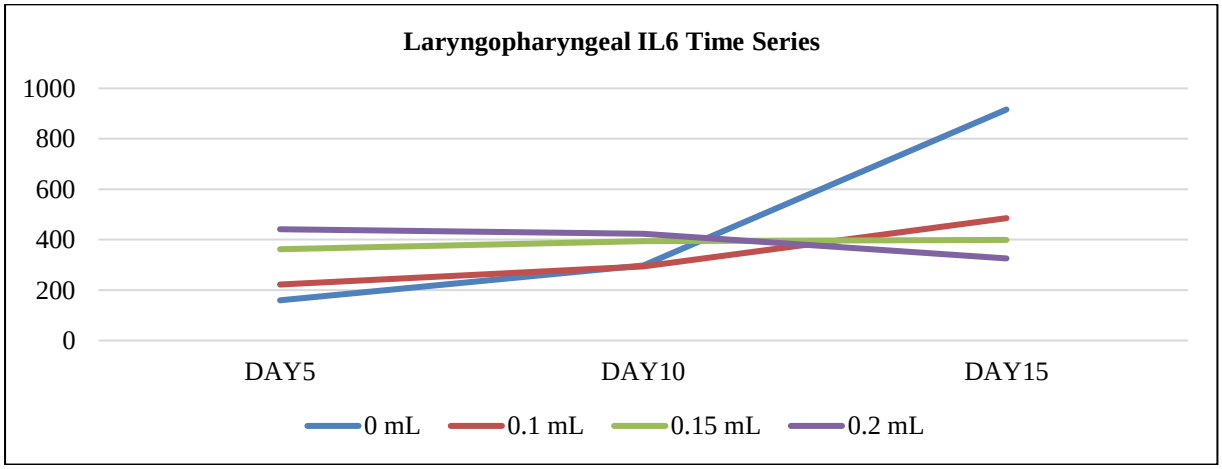

Figure 2. Laryngopharyngeal IL-6 concentration as time series

In contrast to laryngeal IL-6 concentration being primarily affected by volume administered, laryngopharyngeal IL-6 concentration changes were mainly affected by time ($p < 0.001$). Beyond these findings, we discovered significant interaction between day and volume ($p < 0.001$), although administered volume itself did not significantly affect laryngopharyngeal IL-6 concentration ($p = 0.521$). Relevant analysis is presented in Table 6 below.

Table 6. Laryngopharyngeal IL-6 concentration as effect of volume and day

Variable	Partial SS	df	F	*p-value*	*p-value*
Volume	28877.459	1	0.42	0.521	
Day	1834098.3	2	13.25	< 0.001	< 0.001
Day#Volume	1233327.4	2	8.91	< 0.001	
Residuals	3737408.2	54			

Discussion

In our model, we aimed to bypass highly invasive, stressful, and risky pyloric ligation as utilized by Shimazu et al (6). Our study serves a twofold purpose: to create a new model for ligation and to prove the existence of inflammation in said model. Inflammatory processes increase the infiltration of several inflammatory cells in the submucosal region (6). Although laryngeal and pharyngeal regions are generally at approximately neutral pH, gastric content refluxates contain activated pepsin that is able to cause widespread tissue damage (7,8). Exposure to acid can result in the breakdown of epithelial tight junctions (9), which could serve as a possible port-of-entry for pepsin to further aggravate the subepithelial tissues and result in the observed inflammatory changes (10). We aimed to simulate this direct acid-pepsin insult through the direct application of pepsin-gastric juice to the upper aerodigestive tract via the modified IV cannula. Our data suggested the existence of inflammation in the laryngopharyngeal and laryngeal regions after direct application of pepsin-gastric juice to the pharyngeal region. In our model, normal saline served as the baseline inflammation profile of the model. Higher laryngeal and laryngopharyngeal tissue IL-6 concentration was observed in treatment groups (groups D-L, $p = 0.005$ and $p = 0.020$ respectively; Table 3 and Table 5, Figure 1 and Figure 2), which demonstrated the evidence of successful model creation.

Acid and pepsin exposure in laryngopharyngeal reflux also contributes to damage in the mucosal barrier. Epithelial exposure to acid has been known to cause direct tissue injury; on the other hand, pepsin is able to break down the epithelial barrier due to its proteolytic nature (11,12). This tissue injury leads to the release of DAMPs and PAMPs that are recognized by TLRs in monocytes and macrophages. Binding of DAMPs and PAMPs to TLRs results in upregulated Il6, Tnfa, and Il1b transcription through NF-kB-associated pathways to promote tissue repair (13,14). During the inflammatory process, IL-6 is necessary to promote Th2 and Th17 differentiation, monocyte infiltration, and induce the switch between the macrophage M1-M2 phenotypes. During the proliferative phase, IL-6 is also necessary to stimulate cellular migration and proliferation, increase collagen secretion, and promote vascularization (15,16). Moreover, chronic tissue damage can result in a pro-inflammatory environment due to ongoing tissue damage and necroses and bacterial colonization preventing normal cellular migration and proliferation necessary in tissue healing(16). As evidenced in our study, sustained inflammatory responses resulted in a persistent increase of tissue IL-6 in laryngeal and laryngopharyngeal regions in our model.

The differential pattern of inflammation observed between laryngeal and laryngopharyngeal regions are to be expected due to the nature of our model and related rat anatomical features. Opposite to the inferosuperior direction of acid-pepsin flow in clinical laryngopharyngeal reflux (8,17), our model exposed the rat aerodigestive tract in the superoinferior direction. Nevertheless, we were

still able to observe increased inflammatory responses in both laryngeal and laryngopharyngeal regions. The laryngopharyngeal region contains complex structures, including the aryepiglottic fold, which has the potential to harbor a significant amount of irritants for prolonged periods (18,19). Furthermore, the laryngopharyngeal region also marks the transition into the upper digestive tract and air tract, with an associated epithelial transition. The complex structure of the laryngopharyngeal region leads to the region being a common site of tissue injury and trauma and prevents any effective clearance of irritating substances. On the other hand, the pharyngeal region tends to experience direct insult from the exposure of foreign substances; however in this case, relatively smooth mucosal regions of the larynx allow for faster clearance of irritants (18,20,21). As evidenced in our study, we discovered significant impact of the duration of exposure in the laryngopharyngeal region ($p < 0.001$), opposite to that of the pharyngeal region, where the dose serves as the more significant predictor of tissue damage ($p = 0.007$) and evidenced in our time-series graphs.

Laryngopharyngeal reflux in humans is known to cause inflammatory-related tissue changes, including edema, ventricular obliteration, posterior commissure hypertrophy, increased mucus secretion, and tissue granulation (22,23). Human laryngeal and pharyngeal epithelial culture showed evidence of increased proliferation of epithelial cell, presumably as the protective response to pepsin insult (24–26).

The acute-to-chronic transition of inflammatory processes might arise from impaired tissue healing due to a chronic acid-pepsin insult in laryngopharyngeal reflux (11,16,22). Increased tissue pepsin is associated with an increased grade of vocal cord dysplastic leukoplakia and laryngeal nodules, showing a direct relationship between chronic pepsin insult in laryngopharyngeal reflux and dysplastic changes in human studies (27–29).

The chronic laryngeal and pharyngeal changes in chronic pepsin exposure were likely related to the activation of several oncogenes in a prolonged inflammatory environment (25,30). In our model, we provided evidence of prolonged IL-6 secretion, with differential pattern of inflammation in the laryngeal and laryngopharyngeal regions after being exposed to acid-pepsin. Whether the differential pattern of inflammation, as evidenced in our model, is related to the increased susceptibility for dysplastic and neoplastic changes of different regions of the upper aerodigestive tract in the presence of laryngopharyngeal reflux remains open to further exploration.

Despite the evidence of acute inflammation in our model, there are several drawbacks. Firstly, the direct, artificial application of acid-pepsin into the pharyngeal region through the IV cannula is notably different from the physiologic (i.e. inferosuperior) pattern of acid-pepsin reflux. Secondly, our study only measured IL-6 expression across a 15-day period, whereas laryngopharyngeal reflux is known to be a chronic condition that occurs over a much longer timespan. Thus, further studies should continue to explore the different effects of timing on several acute and chronic inflammatory markers and oncogene expressions involved in laryngopharyngeal reflux.

Conclusion

In this study, we demonstrated the feasibility of a non-invasive rat model for laryngopharyngeal reflux through direct application of acid-pepsin into the laryngeal region. We were able to demonstrate the evidence of inflammation due to acid-pepsin exposure in the upper aerodigestive tract. Specifically, we demonstrated dose-dependent inflammatory responses in the laryngeal region and duration-dependent inflammatory responses in the laryngopharyngeal region. This differential pattern of response is likely related to the different pattern of exposure and anatomical differences between the laryngeal and laryngopharyngeal regions.

These findings offer a novel pattern of putative mechanisms underlying regional variation of responses to acid-pepsin insult in laryngopharyngeal reflux for further elucidation in subsequent studies.

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University. The authors are also grateful for the kind assistance from staff of Biomedical Laboratory, Faculty of Medicine, Sriwijaya University.

List of Abbreviations

DAMPs: damage-associated molecular patterns
IV : intravenous
IL-6: interleukin-6
NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B cells
PAMPs: pathogen-associated molecular patterns
Th2: T-helper 2 cells
Th17: T-helper 17 cells
TLRs: toll-like receptors

Authors' Contributions

A conceptualized and conducted animal study, I laboratory study, HD and AS performed data interpretation and analyses. All authors drafted the manuscript.

Data Availability Statements

All data are available upon reasonable request to authors.

References

1. Liu D, Qian T, Sun S, Jiang JJ. Laryngopharyngeal reflux and inflammatory responses in mucosal barrier dysfunction of the upper aerodigestive tract. J Inflamm Res. 2020;13:1291–304.
2. Perotin J-M, Wheway G, Tariq K, Azim A, Ridley RA, Ward JA, et al. Vulnerability to acid reflux of the airway epithelium in severe asthma. European Respiratory Journal. 2022 Aug;60(2):2101634.
3. Brar S, Watters C, Watson N, Birchall M, Karagama Y. Ear, nose and throat (ENT) manifestations and complications of reflux. Frontline Gastroenterol. 2022 Aug;13(e1):e57–64.
4. Lechien JR, Saussez S, Muls V, Barillari MR, Chiesa-Estomba CM, Hans S, et al. Laryngopharyngeal Reflux: A State-of-the-Art Algorithm Management for Primary Care Physicians. J Clin Med. 2020 Nov 10;9(11):3618.
5. Dai Y-F, Tan J-J, Deng C-Q, Liu X, Lv Z-H, Li X-P. Association of pepsin and DNA damage in laryngopharyngeal reflux-related vocal fold polyps. Am J Otolaryngol. 2020 Nov;41(6):102681.
6. Shimazu R, Kusano K, Kuratomi Y, Inokuchi A. Histological changes of the pharynx and larynx in rats with chronic acid reflux esophagitis. Acta Otolaryngol. 2009 Jan 13;129(8):886–92.
7. Rees LEN, Pazmany L, Gutowska-Owsiak D, Inman CF, Phillips A, Stokes CR, et al. The Mucosal Immune Response to Laryngopharyngeal Reflux. Am J Respir Crit Care Med. 2008 Jun 1;177(11):1187–93.
8. Lechien JR, Chiesa-Estomba CM, Henriquez CC, Mouawad F, Ristagno C, Barillari MR, et al. Laryngopharyngeal reflux, gastroesophageal reflux and dental disorders: A systematic review. Vol. 15, PLoS ONE. Public Library of Science; 2020.
9. Lechien JR, Saussez S, Muls V, Barillari MR, Chiesa-Estomba CM, Hans S, et al. Laryngopharyngeal reflux: A state-of-the-art algorithm management for primary care physicians. Vol. 9, Journal of Clinical Medicine. MDPI; 2020. p. 1–18.
10. McConnell EL, Basit AW, Murdan S. Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments. Journal of Pharmacy and Pharmacology. 2010 Feb 18;60(1):63–70.
11. Samuels TL, Johnston N. Pepsin as a causal agent of inflammation during nonacidic reflux. Otolaryngology - Head and Neck Surgery. 2009 Nov;141(5):559–63.
12. Hou C, Zhou L, Zheng Y, Chen T, Hu R, Zheng J, et al. Weak acid and pepsin reflux induce laryngopharyngeal mucosal barrier injury: A rabbit-model-based study. PLoS One. 2025 Jan 1;20(1 January).
13. Narazaki M, Kishimoto T. The two-faced cytokine IL-6 in host defense and diseases. Vol. 19, International Journal of Molecular Sciences. MDPI AG; 2018.
14. Meizlish ML, Franklin RA, Zhou X, Medzhitov R. Tissue Homeostasis and Inflammation. Annu Rev Immunol [Internet]. 2025; Available from: https://doi.org/10.1146/annurev-immunol-061020-
15. Johnson BZ, Stevenson AW, Prêle CM, Fear MW, Wood FM. The role of IL-6 in skin fibrosis and cutaneous wound healing. Biomedicines. 2020 May 1;8(5).
16. Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in chronic wounds. Vol. 17, International Journal of Molecular Sciences. MDPI AG; 2016.
17. Liu D, Qian T, Sun S, Jiang JJ. Laryngopharyngeal reflux and inflammatory responses in mucosal barrier dysfunction of the upper aerodigestive tract. J Inflamm Res. 2020;13:1291–304.
18. Anatomy and Histology of the Laboratory Rat in Toxicology and Biomedical Research. Elsevier; 2019.
19. Riede T, Borgard HL, Pasch B. Laryngeal airway reconstruction indicates that rodent ultrasonic vocalizations are produced by an edge-tone mechanism. R Soc Open Sci. 2017 Nov 1;4(11).
20. Treuting PM, Dintzis SM. Comparative Anatomy and Histology: A Mouse and Human

Atlas. In: Comparative Anatomy and Histology: A Mouse and Human Atlas. Academic Press; 2012. p. 46–51.

21. Mowat V, Alexander DJ, Pilling AM. A Comparison of Rodent and Nonrodent Laryngeal and Tracheal Bifurcation Sensitivities in Inhalation Toxicity Studies and Their Relevance for Human Exposure. Vol. 45, Toxicologic Pathology. SAGE Publications Inc.; 2017. p. 216–22.

22. Stinnett S, Dhillon V, Akst L. Laryngopharyngeal Reflux: Current Concepts on Etiology and Pathophysiology and Its Role in Dysphagia. Vol. 6, Current Otorhinolaryngology Reports. Springer Science and Business Media B.V.; 2018. p. 196–202.

23. Rees CJ, Belafsky PC. Laryngopharyngeal reflux: Current concepts in pathophysiology, diagnosis, and treatment. Vol. 10, International Journal of Speech-Language Pathology. 2008. p. 245–53.

24. Bulmer DM, Ali MS, Brownlee IA, Dettmar PW, Pearson JP. Laryngeal mucosa: Its susceptibility to damage by acid and pepsin. Laryngoscope. 2010 Apr;120(4):777–82.

25. Doukas PG, Vageli DP, Sasaki CT, Judson BL. Pepsin promotes activation of epidermal growth factor receptor and downstream oncogenic pathways, at slightly acidic and neutral ph, in exposed hypopharyngeal cells. Int J Mol Sci. 2021 Apr 2;22(8).

26. Johnston N, Yan JC, Hoekzema CR, Samuels TL, Stoner GD, Blumin JH, et al. Pepsin promotes proliferation of laryngeal and pharyngeal epithelial cells. Laryngoscope. 2012 Jun;122(6):1317–25.

27. Chen YL, Bao YY, Zhou SH, Yao HT, Chen Z. Relationship Between Pepsin Expression and Dysplasia Grade in Patients With Vocal Cord Leukoplakia. Otolaryngology - Head and Neck Surgery (United States). 2021 Jan 1;164(1):160–5.

28. Gong X, Wang XY, Yang L, Sun MJ, Du J, Zhang W. Detecting Laryngopharyngeal Reflux by Immunohistochemistry of Pepsin in the Biopsies of Vocal Fold Leukoplakia. Journal of Voice. 2018 May 1;32(3):352–5.

29. Tasli H, Eser B, Asik MB, Birkent H. Does Pepsin Play a Role in Etiology of Laryngeal Nodules? Journal of Voice. 2019 Sep 1;33(5):704–7.

30. Del Negro A, Araújo MR, Tincani AJ, Meirelles L, Martins AS, Andreollo NA. Experimental carcinogenesis on the oropharyngeal mucosa of rats with hydrochloric acid, sodium nitrate and pepsin. Acta Cir Bras. 2008 Aug;23(4):337–42.