

## PEP TEST AND SOFT TISSUE LESIONS OF THE ORAL CAVITY

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### ABSTRACT

**Introduction:** Gastroesophageal reflux disease (GERD) is a common disorder characterised by signs, symptoms and tissue damage induced by gastric contents. Few studies have investigated the association between soft tissue lesions of the oral cavity and GERD. Pepsin is one of the main components of reflux fluid. It may be found in saliva following reflux episodes. The aim of the study is to evaluate the correlation between the presence of pepsin in saliva and soft tissue lesions of the oral cavity in patients with GERD.

**Materials and methods:** The study was conducted in 100 adult patients with typical GERD symptoms. The salivary pepsin test (PEP test) was used to assess the presence of pepsin in saliva and, therefore, the diagnosis of GERD. The presence of possible soft tissue lesions of the oral cavity was evaluated by means of a dental examination.

**Results:** In 52% of the patients the PEP test was positive. Of these, 40% had soft tissue lesions of the oral cavity. The remaining 48% were negative in the PEP test and only 4% had soft tissue lesions of the oral cavity.

**Conclusions:** The results showed a positive correlation between the presence of pepsin and soft tissue lesions in the oral cavity. However, further investigation is necessary.

**Keywords:** Gastroesophageal reflux disease, GERD, Pepsin, Oral lesions.

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### Introduction

Gastroesophageal reflux disease (GERD) is a common gastrointestinal condition with global distribution and high prevalence in Western countries. The 2006 Montreal Congress defined GERD as a condition that develops when reflux of stomach contents causes distressing symptoms and complications in the oesophagus<sup>(1)</sup>. The attributable symptoms are very debilitating and can be divided into oesophageal (heartburn, regurgitation) or extra-oesophageal (asthma, laryngitis, chronic cough, dental erosions, oral mucosal lesions and non-cardiac thoracic pain). GERD-related tissue damage ranges from oe-

sophagitis to Barrett's oesophagus and oesophageal adenocarcinoma, as extensively demonstrated in the literature<sup>(2)</sup>. Gastric intestinal metaplasia (IM) is expected in the gastric epithelium of patients with chronic atrophic gastritis. A miR-92a-1-5p / FOXD1 / NF- $\kappa$ B / CDX2 regulatory axis plays a key role in generating the IM phenotype from gastric cells<sup>(3)</sup>. MiRs have been linked to the occurrence of oral lesions and periodontitis<sup>(4)</sup>. A careful review of the literature shows that most studies investigating the association between GERD and the oral cavity are limited almost exclusively to hard tissue involvement, while soft tissue involvement remains under-examined to date<sup>(5)</sup>.

A study by Vinesh et al. shows that the most common signs of oral lesions caused by GERD are, in order of highest incidence, dental erosion, periodontitis, gingivitis, gingival and palatal erythema, gingival ulcers, glossitis, erythema of the floor of the mouth and, finally, erythema of the tongue<sup>(6)</sup>.

Moreover, GERD is related to several otolaryngologic disorders such as chronic dysphonia, recurrent otitis media or sense of pharyngeal foreign body<sup>(7,8)</sup>.

The process of chronic inflammation due to damage to the tissues of the oral cavity and the oropharynx also represents a risk factor for the development of neoplasms<sup>(9)</sup>.

Silva et al. analysed the histopathology and morphometry of biopsy sections from the palatal mucosa of patients with a histological diagnosis of GERD. They found that severe GERD subjects had more epithelial atrophy than controls, although the number of fibroblasts was increased. This result represents the reactivity of the oral mucosa in response to local irritants and shows that palatal mucosal changes are only present in subjects with severe reflux<sup>(10)</sup>. There is no gold standard for GERD diagnosis; only 40% of cases are endoscopically positive. In contrast, the proton pump inhibitor test (PPI Test), despite being the most widely used, has been found to have low specificity, as it is based on a reduction in symptoms of at least 50%.

An objective diagnostic instrument with sensitivity, specificity and non-invasiveness remains an unmet need for clinicians and researchers. In dentistry, various diagnostic means are used and exploited when planning the treatment plan. Instrumental, laboratory and photographic examinations are widely used in oral surgery, orthodontics<sup>(11)</sup>, periodontology and endodontics<sup>(11, 12)</sup> but are of little use in the management of soft lesions of the oral cavity, especially if induced by GERD<sup>(13-15)</sup>. Pepsin, secreted by the main cells of the gastric mucosa and activated in acidic gastric secretions, is one of the main components of reflux fluid and a major factor in injury in patients with GERD. Pepsin is deactivated at pH 7.0 and reactivated after acidification. When reflux occurs, pepsin can enter the oral cavity with the reflux fluid and be mixed into the saliva. Therefore, pepsin found in saliva could be a promising diagnostic biomarker<sup>(16)</sup>. As shown in the study by Yan-Jun Wang et al., the detection of pepsin in saliva has an important value for the diagnosis of GERD and GERD-related disorders, but there is still no unanimous consensus on the normal values, sensitivity,

and specificity to be used as a clinical tool for the diagnosis of GERD. It needs further testing before it can become a routine diagnostic tool for patients suspected of GERD<sup>(17)</sup>. Previous studies have shown that tissues in the oesophagus and throat were sensitive to pepsin, and a small quantity of activated pepsin could cause inflammation in the mucosa of the oesophagus and throat, leading to discomfort and subjective symptoms. This enzyme seems to be involved in the occurrence of various lesions of the oesophageal tract and, therefore, in our opinion, also a possible cause of damage to the hard and soft tissues of the oral cavity, leading to mucosal hyperactivity of the upper airways and an increased risk for several respiratory comorbidities<sup>(18)</sup>. However, there is still not enough evidence to state with certainty the correlation between pepsin and lesions of the soft tissues of the oral cavity. The aim of this work is to determine the possible correlation between PEP test positivity and soft tissue lesions in the oral cavity.

## Material and methods

The study was conducted in the Otolaryngology Clinic of the Gaspare Rodolico Hospital from 1 January 2019 to 1 February 2020. One hundred adult patients with retrosternal burning, aged between 18 and 70 years, were examined. The salivary pepsin test (PEP test), a minimally invasive test produced by RD Biomed, which detects pepsin in saliva, was used as a diagnostic tool for GERD. Therefore, the kit allows the presence of pepsin in clinical samples to be identified quickly and easily (approximately 15 minutes after execution). No special preparation is required for use. Approximately 2/3 cc of saliva was taken from each patient under the same standard conditions: in the morning, they woke up. Patients were recommended not to brush their teeth, have breakfast, not smoke, drink coffee, and carbonated substances before the test. Subsequently, the patients underwent a dental examination to assess the possible presence of hard and soft tissue lesions of the oral cavity. The same dentist performed the dental examination under standard conditions.

## Results

52% of the PEP tests performed were positive, leading to the diagnosis of GERD. Preliminary objective examination showed that out of 52 GERD patients, 30% (16 out of 52) had dental erosions; 20% (10 out of 52) had clinically detectable soft tissue

lesions such as periodontitis, gingivitis, erythema of the palate, glossitis; 20% (10 out of 52) had dental erosions and soft tissue lesions and 30% (16 out of 52) had no lesions. Performing the same objective examination on the 48 healthy subjects showed that only 8% (4 out of 48) had dental erosions, 4% (2 out of 48) had soft tissue lesions, and 88% (42 out of 48) had no lesions.

## Discussion

The diagnosis of oral lesions could be very difficult due to the presence of numerous comorbidities described in the literature, both in adult and younger patients<sup>(19-23)</sup>.

Several authors claimed that gastroesophageal reflux disease is highly related to obstructive sleep apnea disorders and severity AHI index is directly proportional to the symptoms' graveness (17,18).

In soft tissue lesions, the differential diagnosis should exclude several disorders such as neoplasms<sup>(24-27)</sup>.

A strong causal link is also reported by several authors between the development of oral soft tissue lesions and various methods of oral palatal surgery till to robotic approach<sup>(27-31)</sup>.

In accord with the literature, the results showed that patients with GERD are more susceptible to developing hard and soft tissue lesions in the oral cavity than healthy subjects<sup>(5)</sup>. Indeed, out of 52 GERD patients, 36 developed oral lesions, 20 of which were soft tissue lesions. In contrast, of the 48 healthy patients, only 6 had oral lesions, including two soft tissue lesions. Besides, the different locations of the lesions lead us to believe that they are due to different aetiological causes: in patients with GERD, the lesions are mainly found in the lingual and palatal areas, whereas in healthy subjects, the location is different from subject to subject. Furthermore, soft tissue lesions in GERD patients do not appear to be closely related to plaque level and oral hygiene but rather to gastric juice exposure and reduced salivary flow.

This hypothesis is further supported by a careful analysis of healthy subjects who, in addition to presenting a significantly lower prevalence of lesions than subjects with GERD, show soft tissue lesions often related to a poor level of oral hygiene and, therefore, to a large accumulation of plaque. In a study performed by Joy Sekine et al. in 2017, in which soft tissue damage caused by gastroesophageal reflux was investigated and the role of salivary

flow, it was found that patients with GERD report significantly reduced salivary flow compared to healthy participants. The authors concluded that the onset of gingivitis and periodontitis in patients with reflux might be exacerbated by xerostomia<sup>(19)</sup>. Therefore, xerostomia should be included in the extraesophageal symptomatology of GERD<sup>(5,32)</sup>. Finally, as the results show, there is a positive correlation between pepsin in saliva and clinically detectable soft tissue lesions in the oral cavity. However, the role of pepsin in the occurrence and prevalence of soft tissue lesions needs further investigation.

The study by Silva et al. found that there is a histological relationship between GERD and oral soft tissue lesions. However, it is not possible to state with certainty that clinical mucosal changes manifest tissue alteration of the soft tissues of the oral cavity in patients with severe GERD. Indeed, neither hyperemia nor continuous lesions of the oral mucosa were found in Silva's patients. Therefore, it is not possible to determine whether acid aggressiveness is sufficient to cause clinical manifestations<sup>(10-13)</sup>. Precisely connected with these findings, it cannot be ruled out that PEP-positive patients, who clinically present with healthy mucosa, do not have tissue changes in the oral mucosa. It would be desirable to investigate the relationship between pepsin and soft tissue lesions in the oral cavity, clinically and histologically.

## Conclusion

The study results show a strong correlation between GERD and soft tissue lesions of the oral cavity. By analysing the lesions on the mucosa and gum tissues and the periodontal apparatus, it is evident that the severity of these lesions is strongly associated with the acid and pepsin-rich contents of the gastric juice. In conjunction with a possible diagnosis of GERD, the early detection of these lesions, employing the PEP test, is a decisive aid to hinder the exacerbation of the pathology, as therapeutic manoeuvres could be carried out immediately in order to *restitutio ad integrum* the various compromised anatomical districts.

## References

- 1) Vakil N, Van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *American Journal of Gastroenterology*. 2006; 101(8): 1900-20.

- 2) Boeckxstaens G, El-Serag HB, Smout AJ, Kahrilas PJ. Republished: Symptomatic reflux disease: the present, the past and the future. *Postgraduate medical journal*. 2015; 91(1071): 46-54.
- 3) Li T, Guo H, Li H, Jiang Y, Zhuang K, Lei C, et al. MicroRNA-92a-1-5p increases CDX2 by targeting FOXD1 in bile acids-induced gastric intestinal metaplasia. *Gut*. 2019; 68(10): 1751-63.
- 4) Grassia V, Lombardi A, Kawasaki H, Ferri C, Perillo L, Mosca L, et al. Salivary microRNAs as new molecular markers in cleft lip and palate: a new frontier in molecular medicine. *Oncotarget*. 2018; 9(27): 18929.
- 5) Warsi I, Ahmed J, Younus A, Rasheed A, Akhtar TS, Ain QU, et al. Risk factors associated with oral manifestations and oral health impact of gastro-oesophageal reflux disease: a multicentre, cross-sectional study in Pakistan. *BMJ open*. 2019; 9(3): e021458.
- 6) Vinesh E, Masthan K, Kumar MS, Jeyapriya SM, Babu A, Thinakaran M. A clinicopathologic study of oral changes in gastroesophageal reflux disease, gastritis, and ulcerative colitis. *The journal of contemporary dental practice*. 2016; 17(11): 943-7.
- 7) Galletti B, Sireci F, Mollica R, Iacona E, Freni F, Martines F, Scherdel EP, Bruno R, Longo P, Galletti F. Vocal Tract Discomfort Scale (VTDS) and Voice Symptom Scale (VoiSS) in the Early Identification of Italian Teachers with Voice Disorders. *Int Arch Otorhinolaryngol*. 2020 Jul;24(3):e323-e329.
- 8) Freni F, Galletti B, Bruno R, Martines F, Abita P, Gazia F, Sireci F, Galletti F. Multidisciplinary approach in the removal of post-trauma foreign bodies in the head and neck district: cases report and review of literature. *Acta Medica Mediterranea*, 2019, 35: 405-410
- 9) Sireci F, Cappello F, Canevari FR, Dispenza F, Gallina S, Salvago P, Martines F. The Role of Leptin in Laryngeal Squamous Cell Carcinoma In: Évariste Gilles and Daniel Mickaël eds. *Leptin: Production, Regulation and Functions*. Nova publisher. pp. 167-176
- 10) Silva MAGS, Damante JH, Stipp ACM, Tolentino MM, Carlotto PR, Fleury RN. Gastroesophageal reflux disease: New oral findings. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2001; 91(3): 301-10.
- 11) Perillo L, d'Apuzzo F, Illario M, Laino L, Di Spigna G, Lepore M, et al. Monitoring Biochemical and Structural Changes in Human Periodontal Ligaments during Orthodontic Treatment by Means of Micro-Raman Spectroscopy. *Sensors*. 2020; 20(2): 497.
- 12) Grassia V, Gentile E, Di Stasio D, Jamilian A, Matarese G, D'Apuzzo F, et al. In vivo confocal microscopy analysis of enamel defects after orthodontic treatment: A preliminary study. *Ultrastructural pathology*. 2016; 40(6): 317-23.
- 13) Giudice AL, Leonardi R, Ronsivalle V, Allegrini S, Lagravère M, Marzo G, et al. Evaluation of pulp cavity/chamber changes after tooth-borne and bone-borne rapid maxillary expansions: a CBCT study using surface-based superimposition and deviation analysis. *Clinical oral investigations*. 2020: 1-11.
- 14) Leonardi R, LGA, Ferronato M., Ronsivalle V., Musumeci G., Spampinato C.. Fully automatic segmentation of sino-nasal cavity and pharyngeal airway based on convolutional neural networks (CNNs). *American Journal of Orthodontics and Dentofacial Orthopaedics*. 2021.
- 15) Nucera R, Lo Giudice A, Bellocchio M, Spinuzza P, Caprioglio A, Cordasco G. Diagnostic concordance between skeletal cephalometrics, radiograph-based soft-tissue cephalometrics, and photograph-based soft-tissue cephalometrics. *European journal of orthodontics*. 2017; 39(4): 352-7.
- 16) Du X, Wang F, Hu Z, Wu J, Wang Z, Yan C, et al. The diagnostic value of pepsin detection in saliva for gastroesophageal reflux disease: a preliminary study from China. *BMC gastroenterology*. 2017; 17(1): 107.
- 17) Iannella G, Magliulo G, Maniaci A, et al. Olfactory function in patients with obstructive sleep apnea: a meta-analysis study. *Eur Arch Otorhinolaryngol*. 2021; 278(3): 883-891. doi: 10.1007/s00405-020-06316-w.
- 18) Iannella G, Maniaci A, Magliulo G, Cocuzza S, La Mantia I, Cammaroto G, Greco A, Vicini C. Current challenges in the diagnosis and treatment of obstructive sleep apnea syndrome in the elderly. *Pol Arch Intern Med*. 2020;130(7-8):649-654. doi: 10.20452/pamw.15283.
- 19) Wang Y-J, Lang X-Q, Wu D, He Y-Q, Lan C-H. Salivary Pepsin as an Intrinsic Marker for Diagnosis of Sub-types of Gastroesophageal Reflux Disease and Gastroesophageal Reflux Disease-related Disorders. *Journal of Neurogastroenterology and Motility*. 2020; 26(1): 74.
- 20) Epstein JB, Silverman S Jr, Epstein JD, Lonky SA, Bride MA. Analysis of oral lesion biopsies identified and evaluated by visual examination, chemiluminescence and toluidine blue [published correction appears in *Oral Oncol*. 2008 Jun;44(6):615]. *Oral Oncol*. 2008 ;44(6): 538-544. doi:10.1016/j.oraloncology.2007.08.011
- 21) La Mantia I, Ciprandi G, Varricchio A, Cupido F, Andaloro C. Salso-bromo-iodine thermal water: a nonpharmacological alternative treatment for postnasal drip-related cough in children with upper respiratory tract infections. *J Biol Regul Homeost Agents*. 2018; 32(1 Suppl. 2):41-47.
- 22) Grillo C, La Mantia I, Zappala G, Cocuzza S, Ciprandi G, Andaloro C. Oral health in children with sleep-disordered breathing: a cross-sectional study. *Acta Biomed* 2019; 90(7-S): 52-59. doi: 10.23750/abm.v90i7-S.8661.
- 23) Caltabiano R, Leonardi R, Musumeci G, et al. Apoptosis in temporomandibular joint disc with internal derangement involves mitochondrial-dependent pathways. An in vivo study. *Acta Odontologica Scandinavica*; 2013, 71: 3-4, 577-583, doi: 10.3109/00016357.2012.700060
- 24) Pennisi M, Malaguarnera G, Di Bartolo G, Lanza G, Bella R, Chisari EM, Cauli O, Vicari E, Malaguarnera M. Decrease in Serum Vitamin D Level of Older Patients with Fatigue. *Nutrients*. 2019 Oct 20; 11(10): 2531. doi:10.3390/nu11102531.
- 25) Pacino GA, Redondo LM, Cocuzza S, et al. Primary hemangiopericytoma of the infratemporal fossa. *J Biol Regul Homeost Agents*. 2020;34(2):691-695. doi:10.23812/19-431-L
- 26) Chen AA, Gheit T, Stellin M, et al. Oncogenic DNA viruses found in salivary gland tumors. *Oral Oncol*. 2017;75:106-110. doi:10.1016/j.oraloncology.2017.11.005
- 27) Schwendicke F, Frencken JE, Bjørndal L, et al. Managing Carious Lesions: Consensus Recommendations on Carious Tissue Removal. *Adv Dent Res*. 2016; 28(2): 58-67. doi:10.1177/0022034516639271
- 28) Iannella G, Magliulo G, Di Luca M, et al. Lateral phar-

- ynoplasty techniques for obstructive sleep apnea syndrome: a comparative experimental stress test of two different techniques. *Eur Arch Otorhinolaryngol.* 2020; 277(6):1793-1800. doi:10.1007/s00405-020-05883-2
- 29) Iannella G, Vallicelli B, Magliulo G, et al. Long-Term Subjective Outcomes of Barbed Reposition Pharyngoplasty for Obstructive Sleep Apnea Syndrome Treatment. *Int J Environ Res Public Health.* 2020;17(5):1542. Published 2020 Feb 27. doi:10.3390/ijerph17051542
- 30) Di Luca M, Iannella G, Montevecchi F, et al. use of the transoral robotic surgery to treat patients with recurrent lingual tonsillitis. *Int J Med Robot* 2020; 16(4): e2106. doi:10.1002/rcs.2106
- 31) Watanabe M, Nakatani E, Yoshikawa H, Kanno T, Nariai Y, Yoshino A, et al. Oral soft tissue disorders are associated with gastroesophageal reflux disease: retrospective study. *BMC gastroenterology.* 2017; 17(1): 92.
- 32) Andaloro C, Sati M, Grillo C, Grillo CM, LA Mantia I. Relationship between sleeping difficulties and airway symptoms severity with the health-related quality of life in patients with GERD. *Minerva Gastroenterol Dietol.* 2017;63(4): 307-312. doi:10.23736/S1121-421X.17.02403-5

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