

Correlation between Salivary Epidermal Growth Factor Concentration and the Pharyngeal Features of the Extraesophageal Reflux

Artigo Original

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RESUMO

Introdução: A Doença do Refluxo Gastroesofágico (DRGE) está associada a várias situações extra esofágicas, como laringites e doenças pulmonares. Até mesmo os pacientes que apresentam queixas que pertencem exclusivamente ao segmento laringofaríngeo (refluxo de Extraesofágico - REE) podem ter a sua qualidade de vida severamente afetada. Os sintomas faríngeos mais comuns são pigarro persistente, globus e halitose. Os mecanismos exatos que conduzem a estes sintomas são obscuros. Estudo recente sugere uma deficiência na concentração de EGF salivar associada a laringites de refluxo. A concentração de EGF poderia também estar associada à presença dos sintomas faríngeos. **Objetivo:** Correlacionar a concentração de EGF salivar em adultos com phmetria positiva para refluxo com a presença de sintomas faríngeos de REE. Desenho metodológico: caso-controle. **Material e Métodos:** 29 sujeitos, 16 com sintomas faríngeos de REE e 13 sem sintomas foram analisados. Os sintomas faríngeos considerados foram halitose, pigarro persistente e globus pharyngeus. Pacientes com sinusite, infecções dentárias ou orofaríngeas foram excluídos, assim como os fumantes. A concentração de EGF salivar foi mensurada por teste de ELISA e os resultados comparados estatisticamente com teste paramétrico. Os sujeitos foram divididos em dois grupos: o grupo sintomático com reclamações consistentes de pelo menos dois dos três sintomas e o grupo assintomático que não apresentava nenhum sintoma. **Resultados:** A concentração de EGF salivar média no grupo sintomático foi de 36.27 pg/ml (intervalo de confiança para 95% - 26.8251 a 43.7262) e no grupo assintomático foi de 88.02 pg/ml (intervalo de confiança para 95% de 50.0642 a 126.4287). A diferença entre estes dois grupos foi estatisticamente significativa ($P < 0.001$).

Descritores: DRGE, fator de crescimento de epidérmico, saliva, refluxo extra esofágico, sintomas, faringe

ABSTRACT

Introduction: Gastroesophageal reflux disease (GERD) has been associated to a number of extra esophageal affections, such as laryngitis and pulmonary diseases. Even patients that present complaints pertaining exclusively to the laryngopharyngeal segment (Extraesophageal reflux - EER) may have their quality of life severely affected. The most common pharyngeal symptoms are persistent throat clearing, globus pharyngeus and halitosis. The exact mechanisms that lead to these symptoms are unclear. A recent study has suggested a deficiency in salivary EGF concentration associated to reflux laryngitis. The EGF concentration could be associated to the presence of the pharyngeal symptoms.

Aim: to correlate the salivary EGF concentration in adults with phmetry positive for reflux to the presence of pharyngeal symptoms of EER.

Study design: case-control study. **Material and Methods:** 29 subjects, 16 with pharyngeal symptoms of EER and 13 without symptoms were analysed. The pharyngeal symptoms considered were halitosis, persistent throat clearing and globus pharyngeus. Patients with sinusitis, dental or oropharyngeal infections, and smokers were excluded from the study. Salivary EGF concentration was measured using a commercially available ELISA kit and the results compared statistically with the parametric variance ratio F-test. The subjects were divided in two groups: the symptomatic group with consistent complaints of at least two of the three pharyngeal symptoms, and the asymptomatic group with subjects presenting no symptoms. **Results:** The mean salivary EGF concentration in the symptomatic group was 36.27 pg/ml (95% CI - 26.8251 to 43.7262) and in the asymptomatic group it was 88.02 pg/ml (95% CI of 50.0642 to 126.4287). The difference between these two groups was statistically significant ($P < 0.001$).

Keywords: GERD, epidermal growth factor, saliva, extra esophageal reflux, symptoms, pharynx

INTRODUÇÃO

Gastroesophageal reflux disease (GERD) is caused by an unbalance between the contact of the digestive mucosa with irritating factors and the efficiency of its protection mechanisms. GERD has been known for more than 50 years in its classic digestive form, and it is currently considered one of the most common gastrointestinal diseases ⁽¹⁾. The first report of acid reflux reaching the larynx was made by Cherry & Marguilles, in 1968 ⁽²⁾; however, it was just in the last two decades and, mainly, after the dissemination of the clinical use of fiberscopes to examine the larynx and the pharynx that GERD became a possible co-responsible for a range of pharyngeal and laryngeal diseases ⁽³⁻⁵⁾. This form of GERD received the denomination of Laryngopharyngeal Reflux (LPR) after Koufman, in 1991 ⁽⁵⁾, but it is better known as extra esophageal reflux (EER).

In spite of the progress in the understanding of GERD, there are still many questions about the mechanisms involved in the genesis and maintenance of the disease, and especially of its atypical form, EER. One of the most significant questions is why patients with the same degree of reflux present clinical manifestations of such different magnitudes. It seems logical to assume that a similar protective deficiency in the laryngopharyngeal mucous membranes could render certain individuals more susceptible to reflux.

Although the mechanical and chemical protection factors of the esophagus are already known, little has been studied of these aspects of the larynx and pharynx ⁽²⁾. The larynx lacks most of the defense mechanisms that the esophagus possesses. In recent years, several studies on the contribution of epithelial growth factors for the defense of the mucous membranes have been published ^(7-11,13,14), a recent article by the current authors attempted to study the behavior of one of these organic elements in the larynx of patients with EER ⁽²⁾. A number of studies have reported a decrease in biologically active polypeptides produced both by the digestive tract mucosa and the saliva, such as the Epidermal Growth Factor (EGF), Prostaglandins (PG), Transformation Growth Factor (especially its sub-fraction - TGF2 alpha), in patients with GERD and peptic disease ^(7,8-14). Other studies demonstrate accelerated recovery of lesions of the gastric and esophageal mucosa after the intravenous infusion of EGF, suggesting an important activity of this polypeptide in the epithelial regeneration ^(15,16).

The epidermal growth factor (EGF) is a peptide composed of 53 amino acids and was discovered accidentally by Cohen, in 1961 ⁽¹⁷⁾. This factor has a wide biological action, and is involved in the induction of epithelial growth, in angiogenesis, in the inhibition of the gastric acid secretion and in the acceleration of wound healing. EGF is reduced in the digestive mucosa and in the saliva

of patients with peptic disease and esophagitis, suggesting a possible protective role of this protein ^(2, 14,15). The first study to show a similar behavior of salivary EGF concentration in adults with reflux laryngitis was published in 2004 ⁽²⁾. However, there seem to be no reports of the behavior of salivary EGF in patients with pharyngeal manifestations of EER. The objective of this study is to correlate the salivary EGF concentration in adults with phmetry positive for reflux to the presence of pharyngeal symptoms of EER.

MATERIAL AND METHODS

From June, 2005 to January, 2006, 29 adults were studied, 16 with pharyngeal symptoms of EER, and 13 without pharyngeal symptoms. The subjects were recruited consecutively from the patients with a positive phmetry for laryngopharyngeal reflux (at least one episode of pH below 4 at the upper probe in 24hs) of the Laryngology Clinic of a tertiary teaching hospital, that consented to participate to the study after properly informed on the objectives, methods and potential risks involved.

All participants responded to an extensive questionnaire on the extra esophageal and gastroesophageal symptoms related to GERD, as well as other diseases associated to posterior laryngitis, such as allergic rhinitis, chronic sinusitis, and oral infections.

Patients of both groups (symptomatic and asymptomatic) were matched by gender and age.

Exclusion criteria were smoking, alcohol abuse, exposure to inhaled chemicals, chronic rhinosinusitis, and use of drugs known to alter salivary secretion, such as, anti-histamines, diuretics, beta-blockers, calcium channel blockers or drugs that alter gastric secretion, such as, H2 blockers, proton pump inhibitors and pro-kinetic drugs in the 7 days prior to saliva sampling. Patients presenting with white lesions, papillomas or laryngeal and pharyngeal neoplasms were also excluded.

The pharyngeal symptoms considered were, throat clearing, globus pharyngeus and halitosis. To be considered in the symptomatic group the subjects had to present at least two to the three complaints.

The group with symptoms was composed of 16 patients (4 men and 12 women), with the mean age of 50.25 years (ranging from 25 to 71 years). The asymptomatic group was composed of 13 patients (3 males and 10 females), with mean age of 44.07 years-old (ranging from 25 to 71 years).

Sampling and processing of saliva

All subjects had saliva sampled in the morning after an 8-hour fasting period and were instructed not to use tooth paste or brush their teeth. After washing the mouth with running water to eliminate epithelial desquamation and bacterial debris, the subjects were instructed to remain seated and deposit all the saliva formed during

a 15-minute period in a recipient contained in ice. This whole saliva (WS) was then centrifuged for 10 minutes at a speed of 5000-8000 rpm, for sedimentation of bacteria, epithelial cells, nuclear and other debris. The supernatant was extracted with a pipette and transferred to a test tube with a threaded tap. Samples were labeled alpha-numerically and stored at minus 20°C up to analysis.

Salivary EGF concentration was determined by a commercial kit of ELISA (Quantikine, R & D Systems, Minneapolis, MN, USA), in which a buffered protein base was used as diluting agent. EGF concentration was expressed by the reading of the optical density of the sample compared to the standard curve, as well as the function of total saliva protein concentration. Thus, the changes in concentration of salivary EGF were related to total concentration of saliva proteins, regardless of total salivary volume.

Statistical analysis

Data were statistically analyzed and the groups were compared by the parametric variance ratio F-test with significance level established at $p < 0.05$.

RESULTS

Eight patients in the symptomatic group presented 2 symptoms and 8 patients referred all three symptoms. In the asymptomatic group no patients presented any pharyngeal symptom. The mean salivary EGF concentration of the study group was 36.81 pg/ml with a 95% confidence interval for the mean (CI) of 26.8251 to 43.7262, and the asymptomatic group had a mean of 88.24 pg/ml with a 95% CI of 50.0642 to 126.4287. The difference between these two groups was statistically significant ($P < 0.001$).

The salivary EGF concentration of the studied population is shown in tables 1 and 2.

Table 1 – Age, Gender and salivary EGF concentration in subjects with pharyngeal symptoms of EER (Study Group).

Subject	Gender	Age	EGF concentration (pg/ml)
1	f	54	43.568
2	m	71	62.528
3	f	46	65.72
4	m	46	20.022
5	f	60	14.04
6	f	71	32.368
7	f	25	41.161
8	m	44	52.023
9	f	62	14.672
10	f	36	21.944
11	f	49	47.404
12	f	57	53.444
13	f	49	46.669
14	f	38	14.774
15	m	35	19.254
16	f	61	39.437

Table 2 – Age, Gender and salivary EGF concentration in subjects without pharyngeal symptoms of EER (Control Group).

Subject	Gender	Age	EGF concentration (pg/ml)
1	f	25	60.953
2	m	27	24.933
3	m	39	54.454
4	f	34	49.465
5	m	34	31.008
6	f	48	27.621
7	f	71	76.118
8	f	35	129.898
9	f	47	132.087
10	f	50	139.341
11	f	33	167.597
12	f	62	32.358
13	f	68	221.371

EGF concentration Arithmetic mean =88.24
95% Confidence Interval for the mean =50.0642 to 126.4287

DISCUSSION

GERD is considered the most prevalent gastrointestinal disease of modern times. Symptoms associated with reflux are reported in 3 to 6% of the general population^(1, 17, 20). In the past 12 years, many researchers and clinicians proposed an association between GERD and chronic laryngitis^(2, 4, 6, 11, 14, 20). However, it is still unknown how many episodes of reflux are necessary to produce inflammatory abnormalities and damage to the larynx and pharynx. Studies such as the ones by DELAHUNTY & CHERRY (1968) and KOUFMAN (1991) demonstrated that the application of chloric acid and pepsin to the laryngeal mucosa of dogs cause contact granulomas and laryngeal stenosis, respectively, after some weeks of administration. These and other studies indicate the laryngeal mucosa is very sensitive to chemical substances and suggest that EER, even if intermittent, can cause severe inflammatory lesions in the larynx⁽¹⁴⁾. It is interesting to note that a large number of patients with EER does not have esophagitis or other signs of GERD in the digestive tube⁽⁶⁾. Certainly, the protection mechanisms of gastric and esophageal mucosa play a decisive role in the ability these organs have to support mechanical and chemical aggressions to which they are submitted daily^(8, 15, 22).

Saliva contains many organic and inorganic substances that may protect against physical and chemical assaults and maintain the integrity of the mucosa^(8, 15, 22, 24). Among the biologically active organic factors produced by salivary glands, the epidermal growth factor (EGF), seems to be directly associated with the quick regeneration of oral and digestive epithelia due to its ability to rapidly replicate DNA^(12, 15, 18, 19, 22). We designed this study in order to assess whether there were differences in salivary concentration of

EGF between subjects with different degrees of pharyngeal symptoms. It was evident that the patients with pharyngeal symptoms of EER presented, as a whole, less EGF in the saliva than the subjects without or with slight complaints (36.81pg/ml and 88.24pg/ml, respectively). There are two possible and distinct explanations to these findings. The first one would be that EER causes a reduction in the salivary production of EGF, and the second possibility would be the exact opposite, where patients with primary low levels

of salivary EGF are more prone to develop EER. Further comparative studies of salivary growth factors are necessary to clarify this matter.

CONCLUSION

The EGF concentration in saliva in patients with pharyngeal symptoms of EER was significantly lower than that of subjects without symptoms.

REFERÊNCIAS BIBLIOGRÁFICAS

1. Johnason JF. Epidemiology of esophageal and supraesophageal injuries. *Am J Med* 2000; 108(4A):99S-103S.
2. Eckley CA, Costa HA. Salivary EGF concentration in adults with reflux laryngitis. *Otolaryngol Head & Neck Surg*, 2004; 131(4): 401-406.
3. Cherry J, Margulies S.I. Contact ulcer of the larynx. *Laryngoscope* 1968; 78:1937-40.
4. Costa HO, Eckley CA, Fernandes AMF, Destailleur D, Villela PH. Refluxo gastroesofágico: comparação entre achados laríngeos e digestivos. *Rev Port ORL* 1997; 35(1):21-6.
5. Eckley CA & Costa HO. Manifestações Otorrinolaringológicas da Doença do Refluxo Gastroesofágico. In: Martinez JC. *Afecções Cirúrgicas do Estômago e Intestino Delgado*. São Paulo: Atheneu; 2002. In press.
6. Koufman JA. The otolaryngologic manifestations of gastroesophageal reflux disease (GERD): a clinical investigation of 225 patients using ambulatory 24-hour pH monitoring and an experimental investigation of the role of acid and pepsin in the development of laryngeal injury. *Laryngoscope* 1991; 101(Suppl):1-78.
7. Rourk RM, Namiot Z, Saroziek J, Yu Z, Mccallum RW. Impairment of Salivary Epidermal Growth Factor Secretory Response to Esophageal Mechanical and Chemical Stimulation in Patients with Reflux Esophagitis. *Am J Gastroenterol* 1994; 89(2):237-44.
8. Sonnenberg A, Steinkamp U, Weise A, Berges W, Weinbeck M, Rohner HG, Peter P. Salivary Secretion in Reflux Esophagitis. *Gastroenterol* 1982; 83:889-95.
9. Starkey RH. & Orth DN. Radioimmunoassay of human epidermal growth factor (urogastrone). *J Clin Endocrinol Metab* 1977; 45(6):1144-53.
10. Gray MR, Donneley RJ, Kingsnorth AN. Role of salivary epidermal growth factor in the pathogenesis of Barrett's columnar lined oesophagus. *Br J Surg* 1991; 78:1461-6.
11. Konturek JW, Bielanski W, Konturek SJ, Konturek JW, Oleksy J, Yamazaki J. Distribution and release of epidermal growth factor in man. *Gut* 1989; 30:1194-200.
12. Marcinkiewicz M, Han K, Zbroach T, Poplawski C, Gramley W, Goldin G, Sarosiek J. The Potential Role of the Esophageal Pre-Epithelial Barrier Components in the Maintenance of Integrity of the Esophageal Mucosa in Patients with Endoscopically Negative Gastroesophageal Reflux Disease. *Am J Gastroenterol* 2000; 95(7):1652-60.
13. Sarosiek J & Mccallum RW. Do salivary Organic Components Play a Protective Role in Health and Disease of the Esophageal Mucosa? *Digestion* 1995; 56 (Suppl. 1):32-7.
14. Sarosiek J, Scheurich CJ, Marcinkiewicz M, Mccallum R.W. Enhancement of Salivary Esophagoprotection: Rationale for a Physiological Approach to Gastroesophageal Reflux Disease. *Am J Gastroenterol* 1996; 110:675-81.
15. Itoh M, Joh T, Imai S, Miyamoto T, Matsusako K, Iwai A, Katsumi K, Endo K, Goto K, Takeuchi T. Experimental and clinical studies on epidermal growth factor for gastric mucosal protection and healing of gastric ulcers. *J Clin Gastroenterol* 1988; 10 (Suppl.1):S7-12.
16. Okita K, Karita M, Nakanishi N. Role of Epidermal Growth Factor in Protection and Repair of Gastric Mucosal Injury. *J Clin Gastroenterol* 1991; 13(Suppl.1):S103-8.
17. Cohen S. Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in the new-born animal. *J Biol Chem* 1962; 237(5):1555-62.
18. Anelli W. In: Aspectos perceptivos-auditivos e acústicos da Doença do Refluxo Gastroesofágico. São Paulo; 2002. p. 97. (Tese Mestrado – UNIFESP).
19. Moraes-Filho JPP, Ceconello I, Gama-Rodrigues J, Castro LP. Brazilian consensus on gastroesophageal reflux disease: proposals for assessment classification and management. *Am J Gastroenterol* 2002; 97(2):241-8.
20. Ronkainen JA, Aro P, Storskrubb T. Prevalence of esophagitis and endoscopy-negative reflux disease in a population. A report from the Kalixandra Study. Abstracts of the Digestive Disease Week 2002; S1357:A-269.
21. Helm JF, Dodds WJ, Hogan WJ. Salivary response to esophageal acid in normal subjects and patients with reflux esophagitis. *Gastroenterol* 1987; 93:1393-7.
22. Olsen PS, Poulsen SS, Kirkegaard P, Nexø E. Role of Submandibular Saliva and Epidermal Growth Factor in Gastric Cytoprotection. *Gastroenterol* 1984; 87:103-8.
23. Dumbrigue HB, Sandow PL, Nguyen KT, et al. Salivary epidermal growth factor levels decrease in patients receiving radiation therapy to the head and neck. *Oral Surg Oral Med Oral Pathol* 2000; 89(6):710-6.
24. Korsten MA, Rosman AS, Fishbein S, Shleim RD, Goldberg HE, Biener A. Chronic xerostomia increases esophageal acid exposure and is associated with esophageal injury. *Am J Med* 1991; 90:701-6.
25. Moazzez R, Anggiansah A, Bartlett D, Owen W. Tooth wear saliva and symptoms of GERD: is there a relationship? Abstracts of the Digestive Disease Week 2002 W1164: A-816.
26. Joh T, Itoh M, Katsumi K, Yokoyama Y, Takeuchi T, Kato T, Wada Y, Tanaka R. Physiological concentration of human epidermal growth factor in biological fluids: use of a sensitive enzyme immunoassay. *Clin Chem Acta* 1986; 158:81-90.
27. Johnston D, Hall H, DiLorenzo TP, Steinberg BM. Elevation of the epidermal growth factor receptor and dependent signaling in human papillomavirus-infected laryngeal papillomas. *Cancer Res*. 1999 Feb 15;59(4):968-74.
28. Scioscia KA, Miller F, April MM, Gruber BL. Growth factors in subglottic stenosis. *Ann Otol Rhinol Laryngol*. 1996 Dec;105(12):936-43.
29. Wen QH, Nishimura T, Miwa T, Nagayama I, Furukawa M. Expression of EGF, EGFR and PCNA in laryngeal lesions. *J Laryngol Otol*. 1995 Jul;109(7):630-6.