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Relationship between gastroesophageal reflux disease and Ph nose and salivary: proposal of a simple method outpatient in patients adults

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Abstract: Introduction: The frequency of gastroesophageal reflux disease (GERD) is increasing, in part through easy inspection of the upper digestive tract, but especially for a real spread of the disease as a consequence of modernity, lifestyle, incorrect dietary rules, and stress arising from social norms. It is a common chronic gastrointestinal disorder in Europe and the United States.

Materials and methods: The aim of our study is to highlight a relationship between gastroesophageal reflux disease and salivary pH as evidenced by indicator strips, especially in the outpatient field. Twenty adult subjects (10 males and 10 females) aged between 18 and 50 years (GROUP A)_ were selected. How to control a homogeneous group of 20 patients without GERD, or from any type of allergies (GROUP B) was enlisted.

Results: This method has provided excellent results showing no difference in the measured values compared with the traditional instrumental measurement.

Conclusion: Our study has allowed us to observe a strong correlation between the saliva pH, nasal cavities and the interaction between the two districts, and could be the basis for a diagnosis of GERD especially in primary health care clinics and in the initial stage of the disease.

Keywords: Gastro-esophageal reflux disease GERD; Ph; Chronic rhinitis; Laryngeal-pharyngeal reflux LPR

1 Introduction

This work follows another study published about the relationship between nasal and salivary pH in people with allergies suffering from GERD and normal subjects.[1] In this paper we strive to detect the ratio of patients with GERD to healthy patients. The esophageal diseases and rhinitis are among the main factors that contribute to chronic cough, and their role has been discussed in several studies. Studies on animal models and on humans show that activators of afferent C-fibers located on the esophageal mucosa or nasal passage do not trigger the cough, but the favor when they take inhaled irritants.

These results are consistent with the hypothesis that activation of C-esophageal and nasal fibers contribute to the cough reflex and hypersensitivity observed in patients with chronic cough from gastroesophageal reflux disease (GERD) and chronic rhinitis. The afferent nerves that cause coughing and esophageal activation are probably vagal fibers, C-fibers arising from the jugular ganglion. In addition to their local response that activates at pH <5, the esophageal C-fibers are also sensitive to bile acids. The neurosensory aspects of the nasal area are currently less well known. The increase of sensitivity of the cough reflex was also reported in several patients with GERD or rhinitis who do not have this disorder, indicating that other endogenous or exogenous factors might cause further

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development of chronic cough. In addition, several epidemiological studies have reported increased coexistence of GERD and chronic rhinosinusitis (CRS) [2,4,5]. It has been shown that the reflux is present more frequently in patients with CRS than those without CRS [3]. There is an ongoing discussion of whether there might be an association between these two diseases and, if so, whether the association is causal. GERD is the most common digestive disease in modern society; it has been associated with abnormalities of the larynx and pharynx (LPR). Gastroesophageal reflux is characterized by the spontaneous movement of gastric contents from the stomach into the esophagus and it occurs daily in all human beings: when it is asymptomatic, it does no harm to the esophageal mucosa and is considered physiological; if it is characterized by inflammation of the larynx, cough, rhinitis, laryngeal tissue alterations ulcers and other symptoms, it can cause severe injuries and illnesses leading to epithelial neoplastic degeneration.

Saliva, with its organic and inorganic components, is responsible for the homeostasis of the oral and digestive tract mucous membranes, contributes to protection against the physical and chemical aggression, thereby maintaining the integrity of the mucosa of the oral cavity—which is part of the digestive tract. Many studies have linked the salivary pH and salivary volume abnormalities with symptoms of GERD and LPR.[8] The presence of GERD may also be caused by other factors, including the release of histamine from mast cells [9-11], which may also promote the onset of this disease, by means of a contraction of the lower esophageal sphincter.

Many studies have shown the variation of the pH in the esophagus as a factor in the onset of GERD using the pH meter as a tool to follow this trend also for the whole day. Some authors argue that there is a relationship between the pH of saliva and the volume and value of esophageal pH [7] and have determined the hypopharynx pH directly in the clinic for patients with hoarseness [12]. Also of crucial importance is tentative diagnosis in the clinic when there is no documentation, as is usually done through the patient's medical history. The diagnosis of reflux disease must be absolutely interdisciplinary, because of the multiplicity of organs involved, through the integration of different specialized skills. The first assessment should be directed toward identification of all aspects of reflux disease: the physician must identify the most relevant symptoms, identify symptoms mistakenly attributed to other causes, and rule out reported symptoms that are secondary to other diseases. The existence of reflux can be already known to the patient, thereby assisting the physician in diagnosis, or be at an asymptomatic level in the esophagus and thus more difficult to identify. In the absence of specific or typical symptoms, there may be recurrent and/or persistent breathing problems that tend to become chronic through hypertrophy of the mucous lining of the airways; this is not uncommon when the diagnosis of reflux is considered by an otolaryngologist according to symptoms and observed in examination of the typical signs of chronic inflammation at the oropharynx, nasopharynx, and larynx. The typical symptoms of GERD are largely gastrointestinal in nature, but there are also atypical symptoms become of interest to otorhinolaryngology that are related to two different routes: vagal stimulation of the esophageal wall characterized by persistent cough, ear pain, pharyngeal-laryngeal paresthesia, pain on swallowing; and direct injury to the refluxed acid on pharyngeal-laryngeal mucosa with dysphagia, pain on swallowing, a sense of a pharyngeal foreign body, drooling, sore throat, laryngospasm, episodes of sleep apnea, and catarrhal otitis. The examining physician can then highlight a significant correlation between atypical manifestations of GERD and otorhinolaryngoiatric pathology related to gastro-nasal reflux and those gastroesophageal laryngotracheal symptoms, all phenomena studied by pH-metry using detectors placed in both the proximal and distal esophagus of the pharynx. The esophageal pH-metry is a minimally invasive technique considered by many researchers to be the best method for GERD diagnosis [15]. This method joins the intraluminal impedance that uses electrical conductivity differences between the esophageal wall and the intraluminal content and is independent of pH. Coupling of impedance and pH-metry appears to be the investigation most often used that is influenced by the PPI therapy drugs frequently used by patients.

The most common cause of GERD is the abnormal function of the lower esophageal sphincter (LES), the tone of which is conditioned by a number of factors; its main task is to permit the relaxation after the start of swallowing, and the post-swallowing contraction to prevent the reflux of the food [13]. In recent times many researchers and doctors have found an association between GERD and chronic laryngitis [16-18,20,21], which is the clinical form of the laryngopharyngeal reflux disease (LPR) characterized, depending on the severity of inflammation of the larynx, by small ulcers, larynx lining alterations, subglottic stenosis, up to epithelial neoplastic degeneration.

2 Materials and methods

The present study proposes a simple, accurate, and non-invasive method, to identify, in subjects with allergic rhinopathy, possible GERD that is then selected for further investigation. In particular, it assesses the relationship between GERD disease and variation of pH of the saliva and nasal as determined by a paper indicator; this method is of great use in the outpatient setting allowing a quick non-invasive and accurate survey.

The 20 adult subjects (10 males and 10 females) patients aged between 18 and 50 years; average age 35.3 (min. 18, max. 50) (GROUP A) were selected from users of the outpatient ENT Diagnostics and nasal cytology AIAS (Italian Association Spastic Assistance) Afragola (Naples), with symptoms that possibly indicated GERD. The control homogeneous group of 20 patients without GERD, or without any type of allergy (GROUP B) was enlisted. The inclusion and exclusion criteria are reported in Table 1.

All patients, after completion of assurance of privacy and informed consent, underwent a physical examination; a rhinofibroscopy with rhinofibroscopy XENIA diameter of 4 mm was performed, using disposable sheaths as a means of prevention. Anatomical elements of GERD, such as admissions of a generalized mucositis, oropharynx shaped (map), and especially at the level of the vocal folds, hyperemia inter arytenoid region, were identified. The next day, outpatients fasted, without having drunk nor performed the daily dental toilet to undergo the examination of saliva.

Before performing the determination of the pH of saliva nasalpH was determined by insertion of a map indicator strips (Macherey-Nagel pH interval 1-14) previously moistened with distilled water directly into each nostril of the patient; subsequently a withdrawal of the nasal mucosa cells on the middle part of the inferior turbinate, bilaterally, with nasalscraping® was performed. The cells were placed on a electrostatically charged cytology slide (SuperFrost Plus Menzel - Gläser Thermo Scientific). Staining was then carried out according to the panoptic Pappenheim method (3 min. In the May-Grunwald dye pure; 6 min. In the May-Grunwald to 50%; 1 min. In distilled water; 30 min. In Giemsa 1:10) in solution. The slide was then covered with a 24 x 50 mm # 1 covering and observed under a Nikon Eclipse 200 optical microscope,at 100x magnification in an oil-immersion. For the acquisition of microscope images we used a Nikon DS FI1 camera with the acquisitions images program NIS - Elements D Version 2.30. For the purpose of a statistical evaluation (although the number is small, the possible change in pH of the two districts was evaluated through the Student's *t*-test. The statisticially significant difference was set as $p<0.05$.

To determine the pH of saliva from each patient 2.5 ml of saliva were collected in a short time without any stimulation, having previously rinsed the mouth with water to avoid contamination. A strip of pH 1–14 range Macherey-Nagel map was dipped in the saliva; after a few seconds the coloration indicating its pH value was observed.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

3 Results

The results obtained are shown in Tables 2 and 3 in which we document a significant statistical variation, as far as possible, between the two groups ($p <0.05$). In particular we can detect an average pH of between 9 and 4.9 for nasal pH in saliva for patients in group A. Furthermore, it detects the presence of abundant mucus basophilic cells that appear in proportion to the pH of the oral cavity. The nasal cytology, also documented in this group as a

Table 1: Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
GERD positive anamnesis and diagnosis	Oncological pathology presence
Allergy absence	Antibiotics, cortisonic, PPI, antihistamine, procinetic therapy
Nasal cavity malformations absence	Significant nasal cavity malformations
Dental practice pathology absence	Poor dental hygiene
Bacterial, viral or mycotic primal breath passage infections absence	Bacterial, viral or mycotic primal breath passage infections presence
Non smokers	smokers
Non Antibiotics, cortisonic, PPI, antihistamine, procinetic therapy	Pregnancy

Table 2: GROUP A patients with GERD.

	Nasal Ph	Salivary Ph	Microscopic framework	Notes
	9	5	Granulocytes ++++	Basophilic mucus
	8	6	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	10	4	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	8	6	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	10	4	Granulocytes ++++	Basophilic mucus
	8	6	Granulocytes ++++	Basophilic mucus
	10	4	Granulocytes ++++	Basophilic mucus
	8	6	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	10	4	Granulocytes ++++	Basophilic mucus
	9	5	Granulocytes ++++	Basophilic mucus
	10	4	Granulocytes ++++	Basophilic mucus
	8	6	Granulocytes ++++	Basophilic mucus
Max	10	6		
Min	8	4		
Dev. Stan	0,72	0,66		
Average	9	4,9		
Median	9	5		

minimum persistent inflammation, was characterized by the reduction of positive SIS ciliated cells, an abundant presence of mucous-like cells, and the absence of bacteria and fungi. There were no differences between the two nostrils.

Table 3: GROUP B control

	Nasal Ph	Saliva Ph	microspy	Notes
	8	7	Normal	Normal
	8	7	Normal	Normal
	8	7	Normal	Normal
	8	6	Normal	Normal
	7	6	Normal	Normal
	9	5	Normal	Normal
	8	6	Normal	Normal
	8	7	Normal	Normal
	8	6	Normal	Normal
	8	6	Normal	Normal
	8	7	Normal	Normal
	8	7	Normal	Normal
	8	7	Normal	Normal
	8	6	Normal	Normal
	7	7	Normale	Normale
	8	6	Normale	Normale
	8	6	Normale	Normale
	7	7	Normale	Normale
	7	7	Normale	Normale
	8	7	Normale	Normale
Max	9	7		
Min	7	5		
Dev. Stan	0,73	0,60		
Average	7,85	6,5		
Median	8	7		

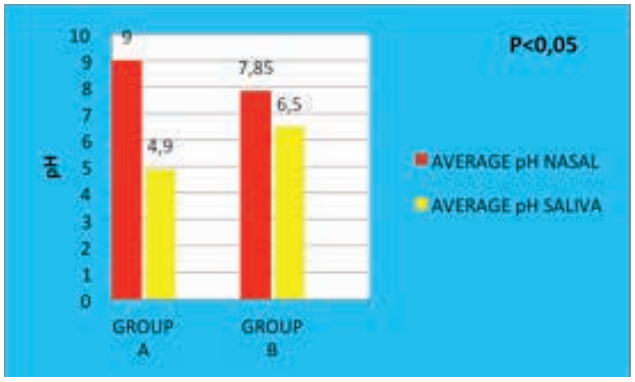

Figure 1: Median pH variation

In group B, there were no interesting pathologies in the mucosa and an absence of basophilic mucus. The nasal pH oscillated between 9 and 7 with an average pH

of 7.85; the pH of saliva ranged between 7 and 5, with an average of 6.5 for the saliva. There were no differences between the two nostrils as examined (Figure 1).

The relationship between sinusitis and GERD studies are in conflict, similar to the relationship of nasal and throat pH. Recent studies, however, seem to confirm the relationship between GERD and diseases of the upper airways, especially with the concentration of pepsin in nasal and laryngeal tissues.

4 Discusssion

The present study aimed to be an approach based primarily on clinical investigations of a deductive type, because it is not always easy to perform a study using a pepsin test. The pH study highlighted in particular a net ratio of the nasal and oral pH; in fact, it was possible to note a trend toward the nasal mucus alkalinity and acidity of the saliva. This allows us to say that whereas the saliva pH, which under normal conditions varies between 7.0 and 7.2, in the presence of GERD is affected by the influence of acid gastric juice, as regards the basicity of the nose, documented, inter alia, both from the study of pH, the nasal cytology, the presence of abundant mucus basic-type cyano coloration in all patients.

This difference between the two pH values is explained by the fact that the nasal mucosa could function as a buffer solution following the acid stimulation of the lower region. The pH is likely physiological, and is associated with the absence of basophilic mucus (which we believe is microscopically pathognomonic of GERD). In this group, the weakly acidic or basic pH can be linked to several factors that do not depend on the reflux, such as the local anatomical variations.

These first results encourage further research on the relationship between the change in the pH in the field of ENT and GERD, especially following the observation of a case with no history of allergy to GERD and borderline pH (8 nasal and salivary 6) with the presence of mucus tends toward the color indication basic pH. Periodic checks in these borderline cases will be needed for a better assessment of the patient's clinical status.

5 Conclusions

Our study has allowed us to observe a strong correlation between the pH of saliva, nasal cavities, and the interaction between the two districts, and could be the basis for a diagnosis of GERD especially in primary health care clinics and in the initial stage of the disease. According to what we saw, to examine a presumptive diagnosis of GERD, we must consider a salivary pH at or below 5; a nasal pH 8 or higher, and the presence of basic nasal mucus. In cases where the pH is borderline, with a mucus tending to slight turn towards the basic, the patient must be rechecked periodically, at least once a month, for further evaluation. The study of salivary vs nasal pH could be used as an element of an additional selection of patients for the presence of pepsin and pH-metry, thereby reducing costs for the NHS (National Health Service). The long-ignored value of salivary analysis has finally been recognized as a valid tool for its diagnostic capabilities: it allows physicians to frequently and easily monitor many diseases that have a strong impact on future research and treatment. At present, we have promising preliminary results that show that saliva can be used to detect lung cancer, pancreatic cancer, breast cancer, and type II diabetes; however, for each disease, further scientific validations are required that compare the diagnostic capacity of saliva as a reference for other body fluids. Salivary diagnostics can play a key role in the routine health monitoring in the near future and enable early detection of diseases using a simple and effective dosage.

Conflict of interest: The authors declare that they have no conflict of interest.

Author contribution

Arturo Armone Caruso: Partecipated substantially in conception, design,and execution of the study and in the analysis and interpretation of data; also partecipated substantially in the drafting and editing of the manuscript.

Salvatore Del Prete: Partecipated substantially in conception, design,and execution of the study and in the analysis and interpretation of data; also partecipated substantially in the drafting and editing of the manuscript.

Lydia Ferrara: Partecipated substantially in conception, design,and execution of the study and in the analysis and interpretation of data; also partecipated substantially in the drafting and editing of the manuscript.

Raffaele Serra: Partecipated substantially in conception, design, and execution of the study and in the analysis and interpretation of data.

Donato Alessandro Telesca: Partecipated substantially in conception, design, and execution of the study and in the analysis and interpretation of data.

Simona Ruggiero: Partecipated substantially in conception, design, and execution of the study and in the analysis and interpretation of data.

Teresa Russo: Partecipated substantially in conception, design, and execution of the study and in the analysis and interpretation of data.

Luigi Sivero: Partecipated substantially in conception, design,and execution of the study and in the analysis and interpretation of data; also partecipated substantially in the drafting and editing of the manuscript.

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