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Laryngopharyngeal reflux: The microbiota theory

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ABSTRACT

Laryngopharyngeal reflux (LPR) is a prevalent disease associated with non-specific symptoms and findings. Many gray areas persist in the pathogenesis of LPR, the diagnosis and the treatment. Symptoms are poorly correlated with fiberoptic signs or hypopharyngeal-esophageal multichannel intraluminal impedance-pH monitoring findings. The therapeutic response remains uncertain with some resistant patients to medical or surgical treatment. The development of LPR-symptoms and findings may be related to the refluxate of a myriad of gastroduodenal enzymes, which may modify the laryngopharyngeal and oral microbiome leading to mucosa maintenance and recovery impairments. The diet of patient is important because it may impact the microbiome composition and some foods are known to increase the number of hypopharyngeal reflux events. The number of hypopharyngeal reflux events may be increased by autonomic nerve dysfunction that may have an important role in the persistence of LPR-symptoms.

Introduction

Laryngopharyngeal reflux (LPR) is a prevalent inflammatory condition of the upper aerodigestive tract tissues related to direct and indirect effect of gastroduodenal content reflux, which induces morphological changes in the mucosa [1]. LPR is associated with many ear, nose and throat conditions, e.g. acute or chronic otitis media [2,3], Eustachian tube dysfunction [4], chronic rhinosinusitis [5], vocal fold benign lesions [6], and non-functional laryngeal disorders [7]. Both symptoms and findings of LPR are non-specific, making the clinical diagnosis difficult [1]. The most prevalent symptoms are throat pain, dysphonia, throat clearing, cough, dysphagia and globus sensation, while the most prevalent findings include oropharyngeal wall erythema, arytenoid erythema and posterior commissure hypertrophy [1]. The hypopharyngeal-esophageal multichannel intraluminal impedance-pH monitoring (HEMII-pH) is the best way to diagnose LPR through the identification of acid, nonacid or weakly acid hypopharyngeal-esophageal reflux events (HRE) [1]. Nowadays, it is well-established that LPR is related to transient esophageal lower and upper sphincter relaxations, and the refluxate of gastroduodenal enzymes, especially pepsin, into the laryngopharyngeal mucosa [1]. Any factor that may impact the esophageal sphincter tonicity, including diet, autonomic nerve dysfunction or anatomical sphincter/esophageal abnormalities, may be involved in the development of LPR [1]. However, scientists are faced with diagnostic and therapeutic controversies and many 'gray' areas related to LPR. Symptoms are not correlated with fiberoptic signs or HEMII-pH findings, and some patients present severe LPR complaints but few HREs at the HEMII-pH [8]. In the same vein, some patients will rapidly respond to treatment, while others will be resistant to any medical or surgical treatment [9]. Currently, the biological mechanisms underlying the development of more or less reflux and the therapeutic responses are still unclear, yielding the management of many patients

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difficult.

Hypothesis

An important factor has been neglected when studying this LPR pathology, that is the fact that the digestive tract is colonized by a huge number of microorganisms. Importantly, bacteria have many functions involved in their ability to grow and colonize the digestive tract whilst bringing plenty of effects for the host that may be beneficial or otherwise. Gut bacteria are able to produce specific enzymes that enable fermentation of nutrients into absorbable forms, including that of indigestible carbohydrates with the production of short-chain fatty acids that may have anti-inflammatory and immunomodulatory effects [10,11]. In addition, it seems that some components of the bacteria themselves may also be released and result in beneficial effects to the host. The interactions between host and gut bacteria are overall important for the maintenance of the gut mucosa and the mucosa recovery [11,12]. The key role of microbiota in the host homeostasis led some authors to investigate its role in the development of many inflammatory diseases of the digestive tube. Thus, microbiota may have a significant role in the development and the symptomatology of ulcerative colitis, Clostridium Difficile colitis, pancreatic, liver, esophageal inflammatory diseases, including esophagitis and Barrett metaplasia [13,14]. In laryngology, the research investigating the potential relationship between laryngopharyngeal microbiota and throat inflammatory diseases are lacking.

Therefore, we hypothesized that the development of laryngopharyngeal mucosa injuries to HREs and the related signs and symptoms are strongly influenced by the laryngopharyngeal microbiota of the patient. In our theory, some patients with altered microbiota could be more susceptible to develop LPR-related symptoms and signs, while others will not develop such findings despite similar number and duration of HREs. In practice, five main elements could interact together in the development of the LPR clinical picture: sensitivity of mucosa; patient diet; autonomic nerve dysfunction, the content of the refluxate and the microbiota.

Sensitivity of mucosa

The human laryngopharyngeal mucosa is a richly innervated structure playing a key role in breathing and swallowing. The sensitivity of laryngopharyngeal mucosa is influenced by many conditions such as genetic pattern, exposition to thermal, mechanical or chemical exposure [15]. It is currently well-admitted that humans are not equal in term of mucosal sensitivity and some patients may more easily develop laryngopharyngeal symptoms when the mucosa is exposed to irritants. It is commonly stated that these individuals have laryngeal hypersensitivity, which is defined as an inappropriate or abnormally heightened response to an external sensory trigger [15]. Interestingly, laryngeal hypersensitivity is suspected as a key condition underlying the development of some laryngeal disorders, i.e. paradoxical vocal fold movements or non-allergic chronic cough, which are moreover associated with LPR [15,16]. According to the mucosa sensitivity, patients with HREs may or may not develop important symptoms and findings. Currently, the origin of the mucosa hypersensitivity is still unknown. In our hypothesis, laryngeal hypersensitivity is a favoring condition of the development of reflux and is related to an altered microbiome. The lack of important bacteria that are involved in the mucosa maintenance and recovery may easier lead to mucosal inflammation and related findings and symptoms.

Diet

Diet is known to be a key factor in the development of LPR through the modulation of the esophageal sphincter tonicity and the related occurrence of HREs [17]. Precisely, low-protein, high-fat, acid and high-sugar diet may reduce the esophageal sphincter tonicity, leading to transient esophageal sphincter relaxations and related HREs at the HEMII-pH [17]. Other foods may impact the gastroesophageal physiology including spicy or industrial foods that contain acidific conservative agents [18,19].

We presume that the diet habits of reflux patients influence the gut microbiota and the related promotion of some bacteria with specific downstream phenotypic features in the host. Thus, we may hypothesize that some foods, which may be partially metabolized in the oral cavity, could impact the oral and laryngopharyngeal microbiome. In gut microbiota studies, it has been showed that individuals consuming more meat have significantly different gut microbiomes to those with plant-based diet [20]. The high consumption of meat, rich in saturated fats but low in unsaturated fats, may lead to a higher probability to develop some inflammatory diseases, which is not the case in patients with plant-based diet [21]. Interestingly, the benefit of plant-based diet has been also reported in LPR patients [19,22,23]. The benefit of plant-based diet in LPR is based on the low composition of saturated fat and the presence of antioxidants. However, vegetable and high-fiber foods take a longer time to be digested into the stomach, leading to a higher risk of transient relaxations of lower esophageal sphincter [18]. In this regard, the microbiota theory may provide an additional hypothetical response to the benefit of plant-based diet: vegetable and high-fiber foods could be beneficial for the esophageal, laryngopharyngeal or oral microbiome and the related mucosa recovery and maintenance.

Autonomic nerve dysfunction

Western populations are characterized by a high level of stress and anxiety that are associated with autonomic nerve dysfunction. The sympathetic and parasympathetic balance is important for the esophageal sphincter tonicity and a dysregulation in favor of the sympathetic system may lead to sphincter relaxations, HREs and LPR [24,25]. The autonomic nerve system is also involved in the functioning of the digestive tube through the gut microbiota-brain axis, which includes gut microbiota and their metabolic products, enteric nervous system, sympathetic and parasympathetic branches within the autonomic nervous system, neural-immune system, neuroendocrine system, and central nervous system. In our hypothesis, patients with severe or resistant LPR may have stronger autonomic nerve dysfunction, which could not only impact the esophageal sphincter tonicity but also the esophageal microbiome. However, the opposite may be possible: microbiome modifications related to reflux may negatively impact the autonomic nervous system (axis), which may worsen the reflux.

Type of refluxate

Currently, pepsin was identified as the main 'irritating enzyme' for the laryngopharyngeal mucosa, leading to the development of PepTest® (RD Biomed Ltd., Hull, United Kingdom) that provides a pepsin concentration value [1]. However, saliva pepsin is not detected in a substantial number of patients with demonstrated LPR at the HEMII-pH. The sensitivity and the specificity of this approach are 64% and 68%, respectively [26]. Moreover, the correlation between saliva pepsin concentration, symptoms, signs and HEMII-pH findings is poor and often not significant [27,28]. Interestingly, LPR patients may have elevated levels of saliva bile salts [29] and bile salts may be involved in the development of laryngopharyngeal carcinoma *in vitro* [30].

In our theory, we propose that pepsin is not the only enzyme that may irritate the digestive mucosa. Bile salts, bilirubin, trypsin, lipase and elastase may theoretically induce laryngopharyngeal mucosal chemical injuries as well as impacting the microbiome. Bicarbonate and pH are interesting markers to characterize the saliva. Depending of the type of refluxate and the proportion of each enzyme, patient may have a selection process of the bacteria composing the laryngopharyngeal and oral microbiome, leading to specific clinical picture of the disease. According to the pattern of the refluxate, some treatments based on

microbiota modulations (e.g. probiotic) could be prescribed to patients, limiting the over-use of proton pump inhibitors that are known to impact the esophageal microbiome [31].

Evaluation of the hypothesis

The first step consists of the clinical evaluation of LPR symptoms and findings through validated instruments. Symptoms may be assessed through Reflux Symptom Score that is a validated patient-reported outcome questionnaire assessing both frequency and severity of LPR-symptoms [32]. The videolaryngological examination may be recorded to evaluated findings by a jury of experienced laryngologists in a blind manner.

Second, patients may benefit from 24-h HEMII-pH (day 0, 8:00 A.M. to day 1, 8:00 A.M.) and collection of saliva samples during this period in order to measure gastroduodenal enzymes through ELISA approach and other biochemical markers through colorimetric and spectrophotometric determination. The saliva sample in the morning of day 1 (fasting) may be used to analysis the microbiome of the patient and to correlate the microbiome findings with the following outcomes: clinical data, refluxate enzymes, HREs nature (liquid, gaseous, mixed) and the pH (acid, weakly acid or nonacid), number and duration of HRE and the diet of the patient. Precisely, the day of the testing, patients may provide precise information about the foods and beverages consumed during the testing period and over the previous days. The refluxogenic potential of the patient usual diet, the diet over the previous month and during the period testing may be assessed through three refluxogenic scores that were previously developed to study the impact of diet on the development of LPR [17,18].

Third, many patient-reported outcome questionnaires exist to subjectively evaluate both anxiety and daily life stress of individuals [33,34]. The objective evaluations of autonomic nerve dysfunction may be performed through several cardiovascular evaluations [24,25], or with Sudoscan®.

Fourth, fibreoptic endoscopic evaluation with sensory testing is one of the most popular and widely adopted means used to evaluate the laryngopharyngeal sensitivity. This approach involves applying puffs of air, controlled for duration, directly to the laryngopharyngeal walls [35]. This approach has to consider age of patients because there were apparent age-related changes in supraglottic and pharyngeal sensory discrimination, with those under 60 having a statistically significant lower sensory discrimination threshold [36].

To be effective, only patients with LPR (HRE $>$ 1) and without confounding conditions (e.g. laryngopharyngeal allergy, chronic rhinosinusitis, tobacco or alcohol consumption, rhinitis or recurrent ear, nose and throat infections, xerostomia post-radiation, malignancy) have to be included. Similar exclusion criteria have to be considered for the control group where individuals have to benefit from similar evaluations.

Conclusion

Laryngopharyngeal reflux disease may prove to be one of the 21st century's most prevalent diseases. Understanding the pathogenesis of the disease may revolutionize the LPR research, diagnostic and treatment space over the next few decades. The potential identification of both specific enzymatic patterns and microbiome alterations may lead to the development of more individualized treatment plans through the use of specific microbiota modulation strategy on a short to long-term basis regarding the patient requirement. Furthering our understanding of autonomic nerve dysfunction, laryngopharyngeal hypersensitivity and how microbiota may play a role in LPR set the stage for advanced research in a prevalent disease that, to date, has only been explored superficially.

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Declaration of Competing Interest

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