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A Clinical Study of Oropharyngeal Microbial Flora in Laryngopharyngeal Reflux Disease.

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Research Article

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ABSTRACT

Laryngopharyngeal reflux (LPR) refers to retrograde flow of gastric contents to the upper aero-digestive tract which causes a variety of symptoms such as cough, hoarseness and asthma. In this study, we observed the changes seen in the microbiological flora in the pharyngeal wall mucosa in patientswith laryngopharyngeal reflux. A prospective study was conducted on 35 patients with Laryngopharyngeal reflux disease (LPRD) and 15 control patients in the ENT department in a tertiary care hospital. Swabs were taken from all the patients from the posterior pharyngeal wall and sent for microbiological analysis. Among the 35 patients with LPRD symptoms, 14.29% patients had normal pharyngeal flora, 21.88% isolates were of Non-fermenting Gram negative bacilli (*Acinetobacter* and *Pseudomonas* species), while 26.67% isolates were having Gram positive bacteria – (*Staphylococcus aureus* – 70% isolates, *Enterococcus species* – 20% isolates, *coagulase negative Staphylococcus aureus* – 10%). 50%of patients had isolates from the group of Enterobacteriaceae (*E.coli* – 12.5%, *Klebsiella species* – 37.50%, *Citrobacter*– 6.25%; *Enterobacter species*– 43.75%). The presence of bacterial flora in the posterior pharyngeal wall increases with the presence of laryngopharyngeal reflux disease with the predominant organism belonging to Enterobactericeae group.

INTRODUCTION

"The term *reflux* (derived from the Latin words *re*["back"] and *fluere*["to flow"]) literally means *backflow*".The term *gastroesophageal reflux* (GER) refers to the backflow of stomach contents into the esophagus. [1] Laryngopharyngeal reflux(LPR) is a condition where retrograde flow of gastric contents to the upper aerodigestive tract occurs, which may cause a variety of symptoms such as cough, hoarseness, asthma etc [2]. Other terms to describe this condition are extraesophageal reflux disease (EERD), atypical reflux, & supraesophageal (or supra-esophageal) reflux [1].

This condition is causing growing concern among the Indian population off late as more and more patients come with complaints of GERD and LPR. Atypical reflux has a different symptomatology compared to classical GERD, mainly due to the fact that it is extra-oesophageal symptoms. Classically, it is described as typical if it is characterisedby the classic digestive symptoms (pyrosis, regurgitation, epigastric pain) and atypical if the symptoms are with respect to the larynx, pharynx or respiratory airway (cough, globus, dysphagia, etc.) [3].

In some studies done in US, the microbial flora of the normal oropharynx and nasopharynx was evaluated. It was observed that the most common organisms in the normal oropharynx were:

1. *Fusobacterium* [4]
2. HACEK organisms [5]
    - *Hemophilus,*

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- Actinobacillus actinomycetem comitans,
- *Cardiobacterium hominis,*
- *Eikenella corrodens,*
- *Kingella*

3. *Actinomyces*

Other organisms like *Staphylococcus*, *Corynebacterium* and *Bacteroides* have also been identified in other studies[6].However, whether they become altered in the event of injury to the mucosa during laryngopharyngeal reflux, or whether they multiply in the acidic pH created by the stomach acids is not quite clear.

MATERIALS AND METHODS

In our study, 35 patients having atypical symptoms of LPRD were taken as cases and 15 patients were taken as controls. Approval for the study was obtained from the Institutional Ethics Committee.

A thorough history was taken from all the patients. Patient's withpositive history of retrosternal burning sensation, hoarseness of voice, and dysphagiawere grouped as having laryngopharyngeal reflux disease cases and those without positive history as controls. Patients who hadupper respiratory tract infections and those who were on anti-reflux medication (PPIs, Sucralfate etc.) were excluded from the study. A detailed ENT examination was done. A culture swab was taken from the posterior pharyngeal wall from all the patients.The patient was in sitting position, and was asked to keep the mouth open. Using a sterile swab, a swab was taken from the posterior pharyngeal wall by making sweeping movements along the width of the lower portion of the posterior pharyngeal wall, taking care not to touch the swab to the surrounding structures (lateral pharyngeal wall, base of tongue, uvula, tonsillar fossa, anterior & posterior pillars). The swab was then placed in the BHI medium (Brain Heart infusion medium)[7]and sent to our microbiology laboratory for isolation and determination of organisms.

RESULTS AND DISCUSSION

In our study, we noticed that of the 35 patients that we examined, the majority of patients presenting with LPRD were patients within the 31 to 50 years of age range. The controls had patients in the 21 – 30 as well as 41 – 50 age group.The majority of the patients with LPRD were mainly in the range of 38 to 65. The mean age group for patients with LPRD is 42.14$\pm$9.96.The controls were of the age group of 13 to 61 years of age.

There were 18 males in the cases and 7 males in controls. The females were 17 in cases and 8 in controls.

Comparing the distributions of sex in both the cases as well as controls, we found that there were nearly equal number of females and male patients with LPRD.

Normal pharyngeal florain our microbiology laboratory constitutes *Streptococcus viridians*, α haemolytic *Streptococci*, *Aerobic spore bearing bacilli*, *Diphteroides*, *Coagulase negative Staphylococcus* species. In a few cases, 2 isolates were isolated in the same specimen and in both cases, the bacteria isolated were from the Enterobacteriaceae group of organisms.

Table 1 demonstrates that 85.71% of cases had abnormal flora in the oropharynx. 5 patients had normal pharyngeal flora (14.29%). Of the remaining patients, 7 isolates were of Non-fermenting gram negative bacilli (*Acinetobacter* and *Pseudomonas* species).8 isolates were having gram positive bacteria (*Staphylococcus aureus*– 6 isolates,*Enterococcus species* – 2 isolates, *coagulase negative Staphylococcus aureus* – 1). 15 patients had isolates from the group of Enterobacteriaceae (*E. coli* – 2 isolates, *Klebsiella species* – 5 isolates, 1 *Citrobacter* isolate; 6 *Enterobacter species* isolates) - (Figure 1).

Table 1: Distribution of flora among cases and controls

	Microbial flora isolated		Total
	Normal flora	Abnormal flora	
LPRD cases	5 (14.29%)	30 (85.71%)	35 (100%)
Controls	6 (40%)	9 (60%)	15 (100%)
Total	11 (22.0%)	39 (78.0%)	50 (100%)

Table 2 shows the different floras that were isolated in Laryngopharyngeal reflux disease patients, which we have classified and differentiated above.

On closer evaluation, we found that the predominant group of organisms isolated in the Enterobacteriaceae group were both of the *Enterobacter species* and the *Klebsiella species* (Figure 2).

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Figure 1: Bacterial flora among LPRD patients

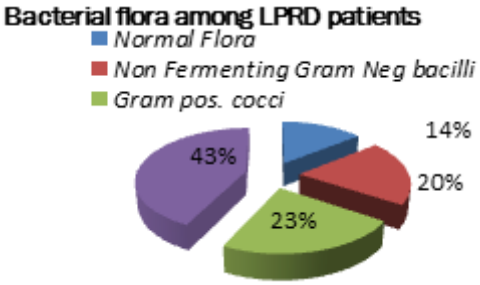


Table 2:- Microbial profile among LPRD patients.

Flora	Frequency (%)
Normal Flora	5(14.29%)
Non Fermenting Gram Neg bacilli	7(21.9%)
Gram pos. cocci	8(26.67%)
Enterobactericeae	15(50%)
Total	35(100%)

Table 3:- Microbial profile among non LPRD patients.

Flora	Frequency (%)
Normal Flora	6(40%)
Non Fermenting Gram Neg bacilli	2(13.33%)
Gram pos. cocci	2(13.33%)
Enterobactericeae	5(33.33%)
Total	15(100.0)

Figure 2:-Microbial profile of *Enterobacteriaceae* group in LPRD patients

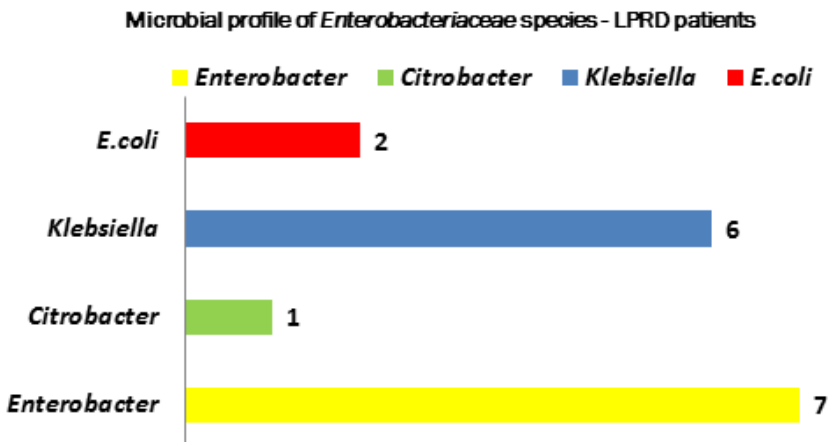
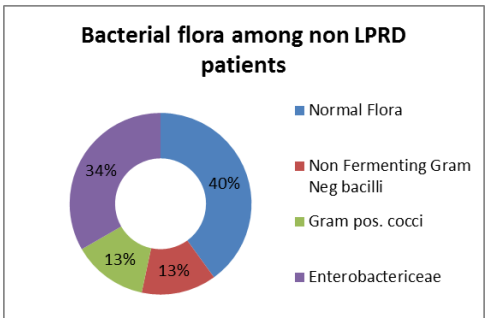


Figure 3:- Bacterial flora among non LPRD patients



Among the 15 controls, we saw the following trends as seen in table 3:

6 patients had normal pharyngeal flora isolated. 2 patients had Non fermenting gram negative bacilli isolated (1 *Acinetobacter* and 1 *Pseudomonas* species), 2 patients with a Gram positive cocci isolated (1 patient *Staph. aureus* and the other patient-*Enterococcus*), 5 patients had been isolated with *Klebsiella* species (Figure 3).

Abnormal flora is higher among LPRD cases (85.71%) compared to non LPRDcontrols (60%) but this difference is not statistically significant, as p value calculated using Fischer's exact test was found to be 0.065 (>0.05).

On evaluating the organisms isolated and broadly classifying them, we found that the patients with LPRD had more of Enterobacteriaceae species in the oropharynx and laryngopharynx, while no other species such as Helicobacter pylori was detected. In comparison, the controls had more patients with normal pharyngeal flora.

Gastro-oesophageal reflux disease and Laryngopharyngeal reflux disease are mainly diseases involving the failure of mechanisms present in the oesophagus to prevent reflux of gastric acid from the stomach to the pharynx. To define each clearly,

- GERD - Gastrooesophageal reflux disease is the abnormal and repeated ascent of gastric contents into the esophagus.
- Laryngopharyngeal reflux disease (LPRD), also called extraesophageal reflux disease refers to retrograde flow of gastric contents to the upper aero-digestive tract, which causes a variety of symptoms.

There are significant differences between GERD and LPRD.

The aetiological and pathological differences for both GERD as well as LPRD vary with each other; for example, GERD occurs more during the night, while LPRD has occurrence in the daytime; GERD occurs in the erect position, while LPRD is more in the supine position; Mechanism of mucosal damage in GERD is due to loss of tone in the lower oesophageal sphincter, while it is loss of upper oesophageal sphincter tone that is affected in LPRD [3]. Both GERD and LPRD are also varied in their symptomatology as well. We know that there are differences in symptomatology, such as epigastric pain, pyrosis, hiccups, acid regurgitation & dyspepsia in GERD. The symptoms in LPRD are pharyngeal globus/dysphagia, dysphonia, sialorrhoea, odynophagia, dry throat, laryngospasm, halitosis, asthma & earache, to name a few [3].

With respect to the pathophysiology of Laryngopharyngeal reflux disease, there are 4 physiological barriers that protect the upper aerodigestive tract [8]:

- The lower oesophageal sphincter
- Oesophageal motor function with acid clearance
- Oesophageal mucosal tissue resistance
- Upper oesophageal sphincter."

Recent investigations have also shown that vulnerable laryngeal tissues are protected by the enzyme carbonic anhydrase. [9]

The delicate ciliated respiratory epithelium of posterior larynx becomes altered when the above protective mechanisms fail and acid reflux occurs into the laryngopharynx. This results in mucociliary dysfunction and mucosal stasis. This mucosal collection will then result in postnasal drip sensation & provokes throat clearing. Direct reflux acid irritation will result in coughing and choking [2].

The above combination of factors results in vocal fold edema, contact ulcers, and granulomas that cause LPR associated symptoms like hoarseness, pharyngeal globus and sore throat [2].

The microbial flora of the oropharynx has not been evaluated properly except in few studies. In a study done by Yarandiet al [10], they showed evidence of higher incidence of GERD in patients with H. pylori infection as compared those patients who did not have H. pylori infection. A study that was done by Gillett et al [11]. Showed that there was overgrowth of normal pharyngeal flora as compared to presence of intestinal or gastric flora in the pharynx. However there are very few studies showing the correlation between the microbial flora and LPRD. In our study, we were able to isolate few intestinal microorganisms in patients with LPRD. The group of bacteria isolated was similar in cases and controls. However patients who had LPRD showed a higher population of the respective bacteria compared to the controls.

With this study we see that presence of abnormal flora is higher among LPRD cases as compared to controls. However this is not significant.

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CONCLUSION

In conclusion, from this study we can see that the presence of intestinal flora is more in the oropharynx in patients with laryngopharyngeal reflux. It is polymicrobial in nature. The patients had a higher population of flora as compared to controls possibly due to the alteration in the pH of the mucosa. We can therefore extrapolate that the presence of bacterial flora in the posterior pharyngeal wall increases with the presence of laryngopharyngeal reflux disease with the predominant organism being Enterobactericeae group.

REFERENCES

1. Koufman JA, Aviv JE, Casiano RR, Shaw GY. Laryngopharyngeal reflux: position statement of the committee on speech, voice, and swallowing disorders of the American Academy of Otolaryngology-Otolaryngol Head Neck Surg. 2002;127(1):32-5.
2. Ford CN. Evaluation and management of laryngopharyngeal reflux. JAMA. 2005;294(12):1534-40.
3. Mosca F, Rossillo V, Leone C. Manifestations of gastro-pharyngo-laryngeal reflux disease. Acta Otorhinolaryngol Ital. 2006;26(5):247.
4. Aliyu S, Marriott R, Curran M, Parmar S, Bentley N, Brown N, et al. Real-time PCR investigation into the importance of Fusobacterium necrophorum as a cause of acute pharyngitis in general practice. J Med Microbiol . 2004;53(10):1029-35.
5. Morpeth S, Murdoch D, Cabell CH, Karchmer AW, Pappas P, Levine D, et al. Non-HACEK gram-negative bacillus endocarditis. Ann Intern Med. 2007;147(12):829-35.
6. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol . 2005;43(11):5721-32.
7. Freundt E. Culture media for classic mycoplasmas. Methods in Mycoplasmology, vol. 1. Academic Press, New York. 1983:127-35.
8. 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(4 Pt 2 Suppl 53):1-78.
9. Axford SE, Sharp N, Ross P, Pearson J, Dettmar P, Panetti M, et al. Cell biology of laryngeal epithelial defenses in health and disease: preliminary studies. Ann OtolRhinolLaryngol. 2001;110(12):1099-108.
10. Yarandi SS, Nasseri-Moghaddam S, Mostajabi P, Malekzadeh R. Overlapping gastroesophageal reflux disease and irritable bowel syndrome: increased dysfunctional symptoms. World J Gastroenterol. 2010;16(10):1232.
11. Gillett SD, Birchall MA, Cogan T, Birchenall K, Rees L. Laryngopharyngeal reflux and the flora of the larynx. Otolaryngol Head Neck Surg. 2009;141(3 suppl 1):66.