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# Pilot study: Association between *Helicobacter pylori* in adenoid hyperplasia and reflux episodes detected by multiple intraluminal impedance in children

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ABSTRACT

*Objectives:* The aim of this pilot study was to investigate an association between laryngopharyngeal reflux detected by combined multiple intraluminal impedance and pH monitoring and *Helicobacter pylori* in adenoid hyperplasia detected with real time polymerase chain reaction (PCR).
*Methods:* The study group consisted of 30 children (median age 5.34 years) with extraesophageal symptoms of gastroesophageal reflux disease with adenoid hyperplasia. All children underwent adenoidectomy with subsequent PCR detection of *H. pylori* DNA in the tissue and multiple intraluminal impedance and pH monitoring. The most proximal impedance sensor was located 1 cm caudal to the entrance of the oesophagus.
*Results:* We found significant differences in the number of reflux episodes among patients with PCR positivity (median 35) and negativity (median 0) of *H. pylori* (*p*-value of Mann–Whitney *U*-test 0.0056). Patients with PCR positivity of *H. pylori* had significantly more reflux episodes reaching the upper oesophageal sphincter (*p*-value of Mann–Whitney *U*-test 0.023). The absence of reflux episode was the only independent factor for PCR negativity of *H. pylori* in the multiple logistic regression model.
*Conclusions:* These results support the hypothesis that reflux episodes reaching the upper oesophageal sphincter may play an important role in the transmission of *H. pylori* into lymphoid tissue of the nasopharynx and thus may contribute to adenoid hyperplasia in children.


## 1. Introduction

*Helicobacter pylori* (HP) is a gram-negative, spiral, microaerophilic and flagellated bacterium that colonises the gastric mucosa. Its prevalence has been estimated to range from 40 to 80% and it varies widely by geographic area, age and socioeconomic status [1]. Immune changes caused by HP infection and its consequences have been thoroughly investigated in the past in the stomach area [2,3]. It is well known that HP infection is acquired in childhood, often before the age of 5 years [4]. The mechanism of transmission remains unclear. There are three possible routes of transmission: the faecal–oral, the oral–oral and the gastric–oral routes. Cytotoxin-associated gene A (CagA) and vacuolating cytotoxin A (VacA) are the most important virulence factors associated with gastric pathogenesis. The CagA protein stimulates cell signalling through the interaction with several host proteins and this leads to an increased release of cytokines group production [5]. VacA protein causes cytoplasmic vacuolation [6]. Two types of signal regions (s1 and s2) and two types of mid-regions (m1 and m2) are known. Strains bearing the vacA s1m1 gene showed higher degrees of gastric colonisation [7]. Possible causal relationships between HP and extra- gastrointestinal diseases have been studied since 1993.

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**Table 1**
Demographic and clinical characteristics of patients.

| | All patients ($n=30$) | Patients with PCR positivity of *Helicobacter pylori* ($n=27$) | Patients with PCR negativity of *Helicobacter pylori* ($n=3$) |
|---|---|---|---|
| Sex, M/F | 20/10 | 18/9 | 2/1 |
| Age in months, median (interquartile range) | 64 (45–78) | 64 (46–77) | 76 (64–78) |
| Symptoms (%) | | | |
| Dysphonia or hoarseness | 12 (40%) | 10 (56%) | 2 (67%) |
| Chronic cough | 11 (37%) | 10 (22%) | 1 (33%) |
| Heartburn | 8 (27%) | 8 (44%) | 0 |
| Other symptoms[a] | 3 (10%) | 3 (17%) | 0 |
| Laryngoscopic findings (%) | | | |
| Laryngitis posterior | 12 (40%) | 11 (61%) | 1 (33%) |

[a] Dysphagia, stridor and globus sensations.

Gastroesophageal reflux (GER) could also be a cause for the enlargement and multiplication of lymphoid follicles [8]. Recent studies have shown that the exposure of upper aerodigestive tract to gastric secretion may cause inflammatory changes that could eventuate into adenoid hyperplasia [9] and the possible role of HP in the pathogenesis of adenoid hyperplasia by gastroesophageal and laryngopharyngeal reflux disease transmission [10]. The nasopharynx area may be directly exposed to the action of HP particularly in patients with gastroesophageal reflux disease (GERD), including diseases arising on the basis of gastroesophageal reflux (GER), as well as laryngopharyngeal reflux (LPR). There are many reports of HP detection in hyperplastic adenoids of children [11–14]; however, the detection rate is highly variable and the assumption that HP can colonise adenoids tissue has not yet been clearly established. In the literature, studies focused on the relationship between the presence of HP in adenoid hyperplasia and LPR in children are limited [15]. LPR is a condition in which the penetration of (duodeno) gastric refluxed fluid above the upper oesophageal sphincter (UES) occurs. We distinguish between physiological LPR, which does not cause any mucosal changes, and pathological LPR, which causes mucosal changes and clinical symptoms. Laryngopharyngeal reflux disease (LPRD) is a condition which causes reflux symptoms and/or complications in the pharynx, oral cavity, larynx and respiratory tract. Prevalence and incidence of LPRD in childhood and adolescence is not exactly known. Mechanisms of reflux in patients with GER and LPR are different. Pathological LPR is regarded as one of the factors which participates in inflammatory processes in the pharynx, larynx and upper respiratory tract. The most important factor in patients with LPR is a dysfunction of the upper oesophageal sphincter (UES) [16]. LPR is most commonly associated with the reflux/posterior laryngitis, vocal cord granulomas, globus pharyngeus, chronic cough and so-called postnasal drip syndrome in childhood [17] . Mucosa of the upper respiratory and swallowing tract in different patients is variously sensitive to aggressive components of refluxed fluid (hydrochloric acid, pepsin and bile acids) and the subjective perception of clinical symptoms is also very individual. We distinguish acid reflux (pH $<4$), weakly acid (pH 4.01–6.9) and non-acid/alkaline (pH $\geq 7$). The question of LPR is full of controversial opinions. There are two opinions still discussed in the pH-metric diagnosis of LPR. One group of authors believes that each episode of LPR is pathological [18,19], while the second group evaluates the LPR pathological if 3–7 reflux episodes occur within a period of 24 h [20,21]. Studies focused on defining the "cut-off" value for the reflux index (RI) are inconsistent, particularly with regards to the age range of the paediatric population. At present, there are no normative value dates that would determine the numbers of LPR as physiological. The aim of this study was to investigate an association between LPR detected by combined multiple intraluminal impedance and pH monitoring (MII-pH) and the presence of HP in adenoid hyperplasia detected with real time polymerase chain reaction PCR.

## 2. Material and methods

### 2.1. Patients

The study included 32 children with the diagnosis of adenoids and symptoms clinically suggestive of LPR for 2009–2012. We suggested enrolment in the study to the parents or legal guardians of children scheduled to undergo adenoidectomy and examination of LPR. The study was approved by the Ethics Committees of the authors' institutions, and a written informed consent was obtained from all participants or their guardians. Clinical characteristics of patients are listed in Table 1.

All patients underwent adenoidectomy at the Department of Ear, Nose and Throat, 2nd Faculty of Medicine, University Hospital Motol, Charles University, Prague, Czech Republic. All 32 patients had confirmed adenoid hyperplasia preoperatively using a flexible endoscopic examination of nasopharynx area. They have also well-known typical symptoms of enlarged adenoids. We did not include patients having tonsillar hypertrophy or any history of chronic tonsillitis in the study. All patients were asked about symptoms of GERD with oesophageal and extraoesophageal symptoms such as dysphonia, chronic cough, hoarseness or regurgitation and *pyrosis*. We evaluated the signs of posterios laryngitis (oedema and erythema of posterior commissure) or other possible LPR findings - pseudosulcus vocalis, ventricular obliteration, diffuse laryngeal oedema and posterior commissure hypertrophy. None of these patients was using anti-reflux therapy or had ever been diagnosed with GERD. However, 2 of these patients (2/32) were unable to be scheduled for MII-pH probe monitoring and were excluded from this study. Therefore, finally 30 patients underwent evaluation for LPR by MII-pH. The introduction of the measuring probe was performed 2 weeks after adenoidectomy, not later than one month.

### 2.2. Detection of HP

#### 2.2.1. Specimen collection and transport

The samples from adenoids were collected using sterile instruments immediately after endoscopically assisted adenoidectomy. All of them were immersed into Microtest R M4RT transport media (Remel Inc., USA) and transported to the laboratory for analysis.

#### 2.2.2. Nucleic acid isolation and storage

Genomic DNA of *H. pylori* was isolated using MagNA Pure Compact System (Tegimenta AG, Rotkreuz, Switzerland), and MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics) protocol, "Total_NA 400_100", with pre-treatment in MagNA Pure Bacteria Lysis Buffer (Roche Diagnostics). Isolated nucleic acid specimens were stored in $-80\,^{\circ}C$.