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Research Article

Role of *Helicobacter pylori* in Nasal Polyp Formation: A Case-Control Study in Tehran, Iran

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ABSTRACT

Background and objective: The etiological factors for nasal polyps include infection, inflammation or an imbalance of a metabolic pathway. This study was designed to compare serum *Helicobacter pylori* antibodies and *H. pylori*–DNAs between cases of nasal polyp and controls (nasal fracture).

Patients and Methods: This case control study was carried out in ENT Department of Rasul Hospital in Tehran (2007-2008), upon nasal polyp tissues in 62 cases and inferior nasal turbinate mucosa in 25 controls. *H. pylori*–DNAs were searched by qualitative polymerase chain reaction (PCR) and serum specific *H. pylori* antibodies (ELISA IgG and IgA). Comparative tests were performed for the 2 groups, and P value < 0.05 was considered as statistically significant.

Results: The mean age of cases and controls were 37.5 ± 13.7 and 31 ± 11.5 years, respectively. *H. pylori*–DNA was found in 32.3% (20/62) of the cases and 4% (1/25) of the controls (P value = 0.005). Serum *H. pylori* antibody (IgA) was found in 14.5% (9/62) of the cases and 4% (1/25) of the controls (P value = 0.27). However, previous immunity (IgG) was higher in 71% of the cases and 32% of the controls (P = 0.001).

Conclusion: *H. pylori* infection may play a key role in the formation of nasal polyps. We recommend the PCR as the best method of searching for *H. pylori* infection. However, from the data obtained in this investigation it could not be determined whether or not *H. pylori* play a pathogenic role. Long-term antibiotics treatment in cases with nasal polyp, especially in cases with severe chronic rhinosinusitis where patients do not respond to surgery or steroids, may be useful. More randomized controlled trial (RCT) studies are necessary to validate the role of *H. pylori* infection in nasal polyp and the effect of antibiotics for eradication of *H. pylori* infection.

Keywords: Nasal polyp; *H. pylori*; Polymerase chain reaction; ELISA

Introduction

Nasal polyps are benign pedunculated masses of nasal or sinus mucosa which affect between 1 and 4% of the population, and are considered to result from chronic inflammation, but the initial or persisting stimulus for the inflammation is not known [1]. Although nasal polyps are well described in terms of cell and cytokine content, the origin of polyps is not understood. The etiological factors associated with the occurrence of nasal polyps include infection, inflammation or an imbalance of a metabolic pathway, such as the arachidonic acid [1,2]. Some studies described the association between nasal polyps and chronic sinusitis. A variety of bacteria and fungi have been cultured from nasal polyps, but approximately 35% have sterile cultures [2].

H. pylorus is a Gram negative bacterium and is the etiologic agent of some gastrointestinal and extra gastrointestinal diseases. Colonization of *H. pylori* has been found in dental plaques, saliva, tonsils, and sinus mucosa [3]. *H. pylori* might play some roles in upper respiratory tract inflammation [4,5]. They were isolated from nasal and maxillary sinus specimens from patients with chronic sinusitis, chronic otitis media with effusion, and adeno tonsilar tissues [6-13]. Recent studies confirmed the presence of *H. pylori* in nasal polyp tissues [14-16].

Although *H. pylori* infection in Iranian population is high [17-20], the etiology and microbial flora of nasal polyps in Iran is not well understood. In this paper, we investigated *H. pylori* serology and *H. pylori*-DNA in resected nasal polyp tissues in a case control study.

Materials and Methods

This case control study was carried out in ENT Department of Rasul Akram Hospital in Tehran (2007-2008) and approved by the Ethical Committee in ENT –Head & Neck Research Center, Tehran University of Medical Sciences.

Cases included 51 adult cases (>12 years) with nasal polyp surgery. Twenty-five normal adults for elective repair surgery (nasal fracture) were selected for controls. Initially, a questionnaire was completed by an authorized physician, followed by complete clinical exams.

All cases and controls were visited by an internist specialist before surgery for other concomitant disorders (immune deficiencies state, diabetes mellitus, renal/heart failure, etc.).

We excluded all cases with immunodeficiency states, diabetes mellitus, and renal failure, patients who received antibiotic at least 2 weeks before surgery, and cases with known malignancy or other diseases proved in pathologic studies.

Blood samples (2 ml) were centrifuged. The serum was restored in -20°C temperature freezer until the serologic examination was performed.

Specific *H. pylori* antibodies (IgG and IgA) were investigated in all cases and controls by ELISA assay. Using the commercial kits (Chemicon-Germany), the results were interpreted quantitatively as suggested by the manufacturer.

During surgery, 1 cm of resected polyp tissue in cases and 1 cm of inferior nasal turbinate mucosa in controls were resected and put down in sterile tube; the samples were centrifuged and homogenized, and the tubes were preserved in -80°C refrigerator.

Purification of the qualitative kit (Roche, Germany) was used for the detection of *H. pylori* -DNA for all prepared tissue samples as manufacturer in "Roche Diagnostics". Polymerase chain reaction (PCR) template Purification Kit (Roche, Germany) was used for all prepared tissue samples. Steps for DNA–Extraction were carried out. The binding column tube was transferred to a new 1.5 ml tube, after which the integrity of the DNA was assessed by gel electrophoresis (1% agarose).

H. pylori-DNAs were searched by qualitative specific PCR primers kits (QIAquickP® QIAGEN; Germany). Diagnostic kits included a ready to use PCR mix kits, positive and negative controls and other qualified reagents along with an easy to follow protocol for detecting as low as 10 copies/ml of *H. pylori* genome.

Statistical Analysis

Student's t test was used to determine significant differences in means for continuous variables and Chi-square was used for comparing categorical data in cases and controls. P-values less than 0.05 were considered as statistically significant.

The agreement between the serologic test and PCR was assessed by the calculation of kappa statistic. Landis and Koch suggested that if a kappa is greater than 0.75, it represents excellent agreement beyond chance, while if a kappa is below 0.40, it represents poor agreement, and if a kappa is between 0.40 and 0.75, it represents intermediate to good agreement.

Results

Demographic results

The people that made up the cases were between 12 and 63 years of age (mean age = 37.5 ± 13.7 years); 63% (39) of the cases were males and 37% (23) were females. The people that made up the controls were between 18 and 25 years of age (mean age = 31 ± 11.5 years).

PCR results

The PCR results show that positive *H. pylori*–DNA in nasal polyp tissues was 32.3% (20/62) and was significantly higher than nasal turbinate tissues in the controls (4%; 1/25) (p-value = 0.01; OR = 11.4) (Figure 1).

Serologic results

The results obtained from the serologic analysis show that *H. pylori*-IgA had no significant difference between cases and controls [14.5% (9/62) of the cases vs. 4% (1/25) of the controls] (p-value = 0.27, or p-value = 4.1)(Figure 2).

Previous immunity against *H. pylori* (IgG) had significant difference between cases and controls [71% (44/62) vs. 32% (8/25)] (p-value = 0.001; or p-value = 5.2)(Figure 3).

We observed the poor agreement between positive *H. pylori*–DNA (PCR) and serum *H. pylori*-IgA antibody (actual agreement = 78.2%; p-value = 0.005; Kappa = 0.27), and positive *H. pylori*–DNA (PCR) and *H. pylori*-IgG antibody (actual agreement = 60%; p-value = 0.001; Kappa = 0.27).

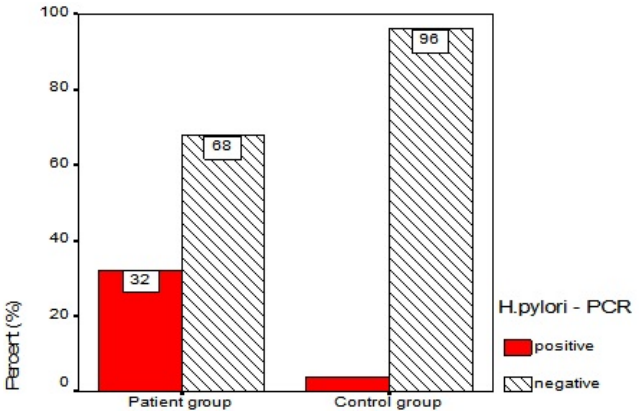

Figure 1: Distribution of *H. pylori* –DNA (PCR) in cases and controls

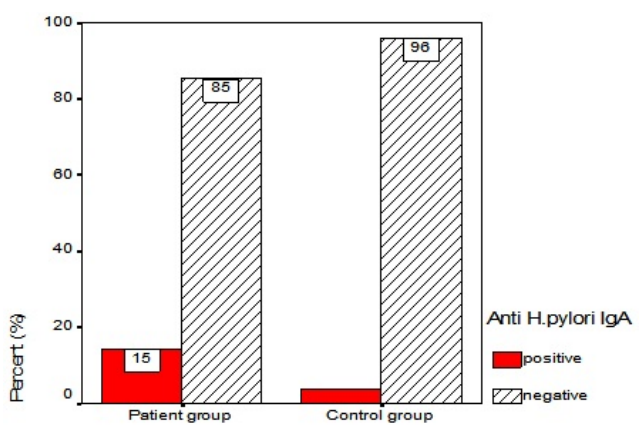

Figure 2: Distribution of *H. pylori* -IgA (ELISA) in cases and controls

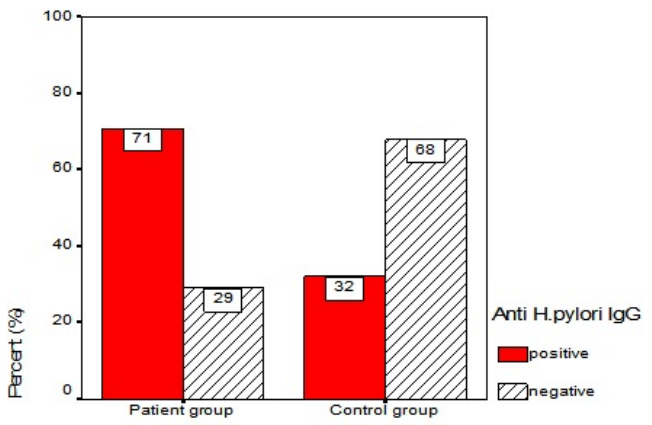

Figure 3: Distribution of *H. pylori* -IgG (ELISA) in cases and controls

Discussion

We defined the higher rate of previous infection with *H. pylori* in the study's cases by at least 2 specific diagnostic tests. Positive *H. pylori*–DNAs were found 8 times more in polyps tissues in comparison with normal tissues. In fact, previous immunity against *H. pylori* (IgG) was at least 2 times more in cases than in controls, but was not true for *H. pylori*-IgA antibodies between cases and controls (p-value = 0.27).

Results of the present study are very close to those of a Turkish study [6] probably, chronic or persistent infection with *H. pylori* occurred in polyp tissues of the studied cases which was higher than healthy controls. However, acute *H. pylori* infection had similar results between cases and controls. These results indicated that 70% of the cases with nasal polyp had previous *H. pylori* infection but only about 32.3% of the infected cases had chronic and persistent infection in nasal polyp (positive-DNA) for a longer period.

We observed poor agreement between *H. pylori*–DNA (PCR) in tissues and positive *H. pylori*–IgA and IgG antibodies in serum (Kappa index = 0.27).

Serum *H. pylori* antibodies (ELISA) tests (IgA and IgM) compared with PCR has lower specificity for diagnosis of local infection in nasal polyp tissues. The positive serology could show the colonization in gastrointestinal tract or other sites and nasal polyp. The specific *H. pylori* culture or DNA of nasal polyp tissue in cases with positive *H. pylori* serology could differentiate the active *H. pylori* in nasal polyp from colonization. Therefore, serologic examinations are not recommended for diagnosis of active infection in cases with nasal polyps. Indeed, there will be false negative culture if the cases received previous antibiotics. In our opinion, testing the *H. pylori*-DNA in nasal polyp tissues is reliable and specific for diagnosis of active *H. pylori* infection. The previous antibiotic usage does not affect the PCR results.

The present results are similar to those of other studies [17-21]. More so, the serologic results are very close to those of other studies done in Iran [17,18]. It was observed that 32% of our controls had previous immunity (IgG) as against *H. pylori* infection. However, sero-prevalence to *H. pylori* infection is high in Iranian population [16,17].

Initial infection probably occurs at an early age, and its prevalence increases with age. Prevalence of the infection increased to 30% in the 2nd decade and 53.5% after the 4th decade of life [16]. *H. pylori*–DNAs in cases with nasal polyps are very close to those of the study of Khademi et al. [10]. *H. pylori* infection was found in tonsil and adenoid tissues of 48.2% of the studied cases (3 to 43 years) by urease test [10], but was 2 times more than that found in the adenoid tissue (*H. pylori*–DNA) of the studied children (with mean age 7.5 years) in our center (32.3% vs 15%). Indeed positive *H. pylori*-IgA and positive *H. pylori*–IgG was reported in 15 and 11% of the children with rhinosinusitis > 2 weeks (mean age 4.2 years) respectively.

Saffari et al. studied *H. pylori* antibodies in the population of Shiraz (south of Iran). Positive *H. pylori*-IgG and IgA were observed in 28.3, 32, 16.7 and 53.5% of persons between 20-40 and 41-80 years of age, respectively [17].

In conclusion, *H. pylori* infection has a high prevalence in Iranian population. Initial *H. pylori* infection might occur at an early age (4 years) in our country, but its prevalence increases with age. The infection increases to 30% in the 2nd decade and 53.5% after the 4th decade of life. Chronic and persistent infections (positive-DNA) were found in parts of the upper respiratory tract (nasal polyp, adenoid hypertrophy) for a longer period. *H. pylori* infection was detected in adenoid tissues of 15% of the children who are not up to 8 years of age and 48% of the adult cases, and in nasal polyp tissues of 32.3% of the studied persons in Iran.

The data presented here are compatible with those presented in other studies, and they show that *H. pylori* can play a possible role in cases with nasal polyps, but its association does not prove causation.

More studies are needed to evaluate this correlation. Nonetheless, placebo - controlled studies should be undertaken before antibiotics are used on a larger scale to treat cases with nasal polyp.

Limitations of the Study

The results define the possible role of *H. pylori* infection in nasal polyps, but its association does not prove causation. Further studies are needed to evaluate the role of *H. pylori* in the etiology of nasal polyps. The search for *H. pylori* infection in nasal polyp by a more specific method, such as Real time-PCR, or specific culture may elucidate better the role of *H. pylori* in nasal polyp in the future.

Conclusion

In this study, the possible role of *H. pylori* infection in nasal polyps was defined and the PCR was recommended as the best method for searching for *H. pylori* infection. However, from the data obtained in this investigation, it could not be determined whether or not *H. pylori* play a pathogenic role. More studies are needed to evaluate this correlation, and future RCT studies are necessary to validate the role of *H. pylori* infection in nasal polyp and the effect of antibiotics for eradication of the *H. pylori* infection.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Bucholtz GA, Salzman SA, Bersalona FB, Boyle TR, Ejercito VS, et al. (2002) PCR analysis of nasal polyps, chronic sinusitis, and hypertrophied turbinates for DNA encoding bacterial 16S rRNA. Am J Rhinol 16: 169-173.
2. Cervin A (2001) The anti-inflammatory effect of erythromycin and its derivatives, with special reference to nasal polyposis and chronic sinusitis. Acta Otolaryngol 121: 83-92.
3. Kurtaran H, Uyar ME, Kasapoglu B, Turkay C, Yilmaz T, et al. (2008) Role of Helicobacter pylori in pathogenesis of upper respiratory system diseases. J Natl Med Assoc 100: 1224-1230.
4. Morinaka S, Ichimiya M, Nakamura H (2003) Detection of Helicobacter pylori in nasal and maxillary sinus specimens from patients with chronic sinusitis. Laryngoscope 113: 1557-1563.
5. Dinis PB, Martins ML, Subtil J (2005) Does Helicobacter pylori play a role in upper respiratory tract inflammation? A case report. Ear Nose Throat J 84: 238-240.
6. Ozdek A, Cirak MY, Samim E, Bayiz U, Safak MA, et al. (2003) A possible role of Helicobacter pylori in chronic rhinosinusitis: a preliminary report. Laryngoscope 113: 679-682.
7. Dinis PB, Subtil J (2006) Helicobacter pylori and laryngopharyngeal reflux in chronic rhinosinusitis. Otolaryngol Head Neck Surg 134: 67-72.
8. Unver S, Kubilay U, Sezen OS, Coskuner T (2001) Investigation of Helicobacter pylori colonization in adenotonsillectomy specimens by means of the CLO test. Laryngoscope 111: 2183-2186.
9. Lukes P, Astl J, Pavlík E, Potuzníková B, Sterzl I, et al. (2008) Helicobacter pylori in tonsillar and adenoid tissue and its possible role in oropharyngeal carcinogenesis. Folia Biol (Praha) 54: 33-39.
10. Unver S, Kubilay U, Sezen OS, Coskuner T (2001) Investigation of Helicobacter pylori colonization in adenotonsillectomy specimens by means of the CLO test. Laryngoscope 111: 2183-2186.
11. Dagli M, Eryilmaz A, Uzun A, Kayhan B, Karabulut H (2006) Investigation of Helicobacter pylori in the middle ear of the patients with chronic otitis media by CLO test and 14C urea breath test. Otol Neurotol 27: 871-873.
12. Skinner LJ, Winter DC, Curran AJ, Barnes C, Kennedy S, et al. (2001) Helicobacter pylori and tonsillectomy. Clin Otolaryngol Allied Sci 26: 505-509.
13. Agirdir BV, Bozova S, Derin AT, Turhan M (2006) Chronic otitis media with effusion and Helicobacter pylori. Int J Pediatr Otorhinolaryngol 70: 829-834.
14. Karlidag T, Bulut Y, Keles E, Kaygusuz I, Yalcin S, et al. (2005) Detection of Helicobacter pylori in children with otitis media with effusion: a preliminary report. Laryngoscope 115: 1262-1265.
15. Koc C, Arikan OK, Atasoy P, Aksoy A (2004) Prevalence of Helicobacter pylori in patients with nasal polyps: a preliminary report. Laryngoscope 114: 1941-1944.
16. Szczygielski K, Jurkiewicz D, Rapiejko P (2007) Detection of H. pylori in nasal polyps specimens using urease test GUT. Otolaryngol Head Neck Surg 136: 390-395.
17. Saffari M, Motavali MA, Fazeli A (2003) Immunoblot Assay in Determination of Serum Antibody Profile of Helicobacter Pylori Infection. Iran J Med Sci 28:90-93
18. Masoodpoor N, Darakhshan, Sheikhvatan M (2008) Helicobacter pylori infection in Iranian children with recurrent abdominal pain. Trop Gastroenterol 29: 221-223.
19. Baghaei K, Shokrzadeh L, Jafari F, Dabiri H, Yamaoka Y, et al. (2009) Determination of Helicobacter pylori virulence by analysis of the cag pathogenicity island isolated from Iranian patients. Dig Liver Dis 41: 634-638.
20. Nouraie M, Latifi-Navid S, Rezvan H, Radmard AR, Maghsudlu M, et al. (2009) Childhood hygienic practice and family education status determine the prevalence of Helicobacter pylori infection in Iran. Helicobacter 14: 40-46.