

## 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  $37.5 \pm 13.7$  and  $31 \pm 11.5$  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.

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**Key words:** Nasal polyp; *H. pylori*; Polymerase chain reaction, ELISA.

**Received** Oct 28, 2015; **Accepted** Dec 28, 2015; **Published** Jan 04, 2016;

## Introduction :

Nasal polyps are benign pedunculated masses of nasal or sinus mucosa which affect between 1- 4% of the population, and are considered to result of chronic inflammation, but the initial or persisting stimulus for the inflammation is not well known<sup>1</sup>. 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<sup>1,2</sup>. 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<sup>2</sup>.

*H. pylori* 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.<sup>3</sup> *H. pylori* might play some roles in upper respiratory tract inflammation.<sup>4,5</sup> They were isolated from nasal and maxillary sinus specimens from patients with chronic sinusitis, chronic otitis media with effusion, and adeno tonsillar tissues.<sup>6-13</sup> Recent studies confirmed the presence of *H. pylori* in nasal polyp tissues.<sup>14-16</sup>

Although prevalence of *H. pylori* infection in Iranian population is high,<sup>17-20</sup> 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.

## Material 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 Research Center, Tehran University of Medical Sciences.

Fifty-one patients (>12 years) with nasal polyp were enrolled in this study.. Twenty-five adult patients for elective repair surgery (nasal fracture) were selected for controls. Initially, a questionnaire was completed by an authorized physician, followed by complete clinical exams.

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 before serologic examination. Specific *H. pylori* antibodies (IgG and IgA) were investigated in all cases and controls by ELISA assay using the commercial kits (Chemicon-Germany). In this method the concentration of antibodies or antigens are evaluated.

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 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. In this sensitive method amplifying the single sequence of DNA or RNA can generate millions copies of a specific DNA or RNA

sequence. 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.

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.

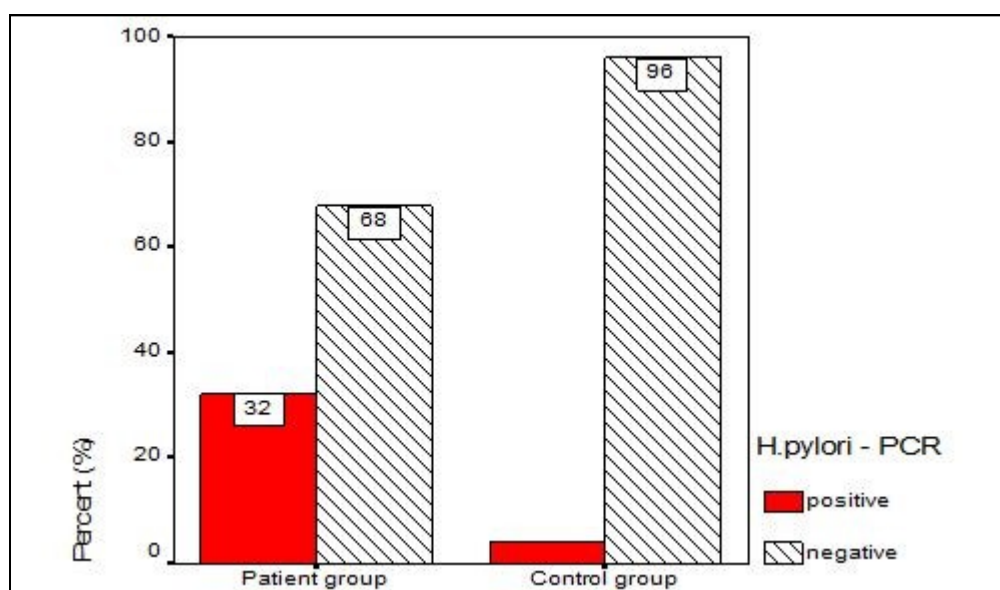
## Results

### Demographic Results

Sixty two cases between 12 and 63 years of age (mean age = 37.5 ± 13.7 years) and 22 controls between 18 and 25 years of age (mean age = 31 ± 11.5 years) were enrolled in this study; 63% (39) of the cases were males and 37% (23) were females.

### PCR Results

The PCR results showed positive *H. pylori*-DNA in nasal polyp tissues in 32.3% (20/62) which was significantly higher than positive results in nasal turbinate tissues in



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

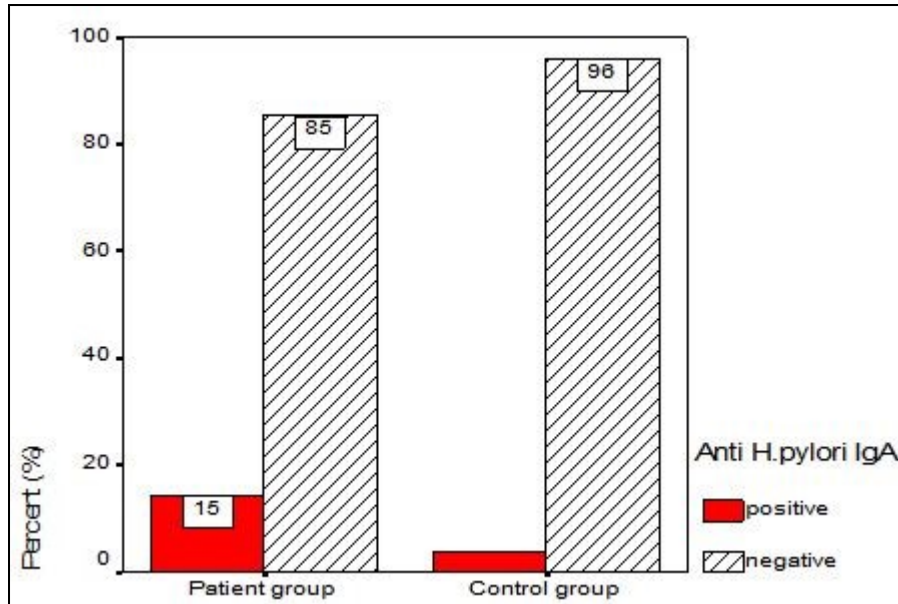
The agreement between the serologic test and PCR was assessed by the calculation of kappa statistic. Landis and Koch suggested that if a kappa is greater than 0.75,

the controls (4%; 1/25) (p-value = 0.01; OR = 11.4) (Figure 1).

### Serologic Results

There was no significant difference of *H. pylori*-IgA between cases and controls [14.5% (9/62) of the cases vs. 4% (1/25) of the controls] (p-value ,0.27 = or

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



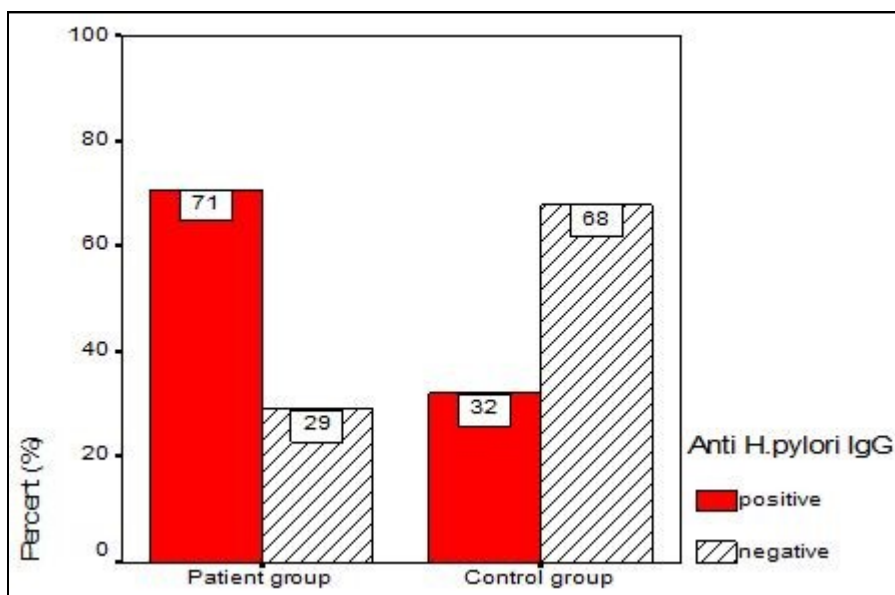
**Figure 2.** Distribution of *H. pylori*-IgA (ELISA) in cases and controls.

p-value = 4.1) (Figure 2).

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

*H. pylori*-IgG antibody (actual agreement = 60%; p-value = 0.001; Kappa = 0.27).

### Discussion :



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

We defined the higher rate of previous *H. pylori* infection in the case group by at least 2 specific diagnostic tests. Positive *H. pylori*-DNAs were found 8 times more in polyps tissues in comparison to normal nasal tissues. In fact, previous immunity against *H. pylori* (IgG) was at least 2 times more in cases than in controls, but similar results was not shown 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.<sup>6</sup> 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 history of *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 presence of *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 identify the colonization in gastrointestinal tract or other tissues such as nasal polyp. Detection of *H. pylori* in culture or DNA PCR 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, *H. pylori*-DNA assay in nasal polyp tissues is reliable and specific for

diagnosis of active *H. pylori* infection because previous antibiotic usage does not affect the PCR results.

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

Primary infection probably occurs at early age, and its prevalence increases with age. Prevalence of the infection increased to 30% in the 2<sup>nd</sup> decade and 53.5% after the 4<sup>th</sup> decade of life.<sup>16</sup> *H. pylori*-DNAs in cases with nasal polyps are very close to those of the study of Khademi et al<sup>10</sup>. *H. pylori* infection was found in tonsil and adenoid tissues of 48.2% of the studied cases (3 to 43 years) by urease test<sup>10</sup>, which 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-IgG werereported in 15 and 11% of the children with rhinosinusitis with more than 2 weeks duration (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.<sup>17</sup>

## Conclusion

*H. pylori* infection has a high prevalence in Iranian population. Primary *H. pylori* infection may occur at early age (4 years) in our country, and increases with age. 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 cases.

The results of recent study are compatible with those presented in other studies.

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 and search for *H. pylori* infection in nasal polyp by a more specific method, such as Real time-PCR, or specific culture are necessary to validate the role of *H. pylori* infection in the etiology of nasal polyps and the effect of antibiotics for treatment of nasal poly via eradication of the *H. pylori* infection.

## ACKNOWLEDGMENT

This study was supported by the ENT –Head & Neck Research Center, Iran University of Medical Sciences

## Conflict of interest

The authors declare no conflict of interest.

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