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Full Length Research Paper

Helicobacter pylori-DNA in nasal polyp tissues in contrast with inferior nasal turbinate tissues: A case-control examination in Tehran, Iran

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This study is aimed at comparing serum *Helicobacter* pylori antibodies and *H. pylori*–DNA in tissue between cases with nasal polyps and controls. A case control study was conducted in the Department of Otorhinolaryngology, Rasul Hospital, Tehran, Persia between 2007 and 2008 upon polyp tissues from 62 nasal polyposis cases and inferior nasal turbinate mucosa from 25 controls. *H. pylori*-DNA was assessed by qualitative PCR and specific H. pylori antibodies (IgG and IgA) were detected in serum by ELISA method and compared between the two groups. P<0.05 was considered significant. The mean age of cases and controls were 37.5±13.7 and 31±11.5 years, respectively. *H. pylori* –DNA was detected in 32.3% (20/62) of nasal polyposis cases and 4% (1/25) of controls (P value<0.005). H. pylori antibodies (IgA) were detected in serum of 14.5% (9/62) cases and 4% (1/25) of controls (P=0.27). Previous immunity (IgG) was observed more often in the serum of nasal polyposis cases (44/62, 71%) vs controls (8/25, 32%) (P=0.001). A possible role for *H. pylori* infection in nasal polyps was defined. We recommend PCR as the best method for evaluating *H. pylori* infection. Whether or not *H. pylori* play a pathogenic role could not be determined from the data obtained in this investigation. If such a correlation is established in future studies, long-term antibiotic treatment in cases with nasal polyp that do not respond to surgery or steroids may be useful

Key words: *H. pylori*, specific *H. pylori* antibodies, *H. pylori* –DNA, Nasal polyp.

INTRODUCTION

Nasal polyps are benign pedunculated masses of nasal or sinus mucosa which affect between 1 and 4% of the population. Nasal polyps are considered to result from chronic inflammation, but the initial or persisting stimulus for the inflammation is not known. 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 (Bucholtz et al., 2002; Cervin, 2001). A variety of bacteria and fungi have been cultured from nasal polyps, but approximately 35% have sterile cultures (Cervin, 2001).

Helicobacter pylori is a gram negative bacterium and considered 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 (Kurtaran et al., 2008). *H. pylori* might have some role in upper respiratory tract inflammation (Morinaka et al., 2003; Dinis et al., 2005). *H. pylori* have been isolated from nasal and maxillary sinus specimens from patients with chronic sinusitis, chronic otitis media with effusion, and adeno-tonsillar tissues (Ozdek et al.,

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2003; Dinis et al., 2006; Unver et al., 2001; Lukes et al., 2008; Khadem et al., 2005; Dagli et al., 2006; Skinner et al., 2001; Agirdir et al., 2006; Karlidag et al., 2005; Bulut et al., 2006; Szczygielski et al., 2007; Saffari et al., 2003; Masoodpoor et al., 2008; Baghaei et al., 2009; Nouraie et al.. 2009; Koc et al., 2004; Ozyurt, et al., 2009; Figura (2011). Recent studies confirmed the presence of *H. pylori* in nasal polyp tissues (Karlidag et al., 2005; Bulut et al., 2006; Szczygielski et al., 2007).

H. pylori infection varies among countries and often within a country. *H. pylori* infection in the Iranian population is high (Dinis et al., 2006; Unver et al., 2001; Lukes et al., 2008; Khadem et al., 2005; Dagli et al., 2006; Skinner et al., 2001; Agirdir et al., 2006; Karlidag et al., 2005; Bulut et al., 2006; Szczygielski et al., 2007; Saffari et al., 2003; Masoodpoor et al., 2008; Baghaei et al., 2009; Nouraie et al.. 2009). Khadem et al. (2005) reported *H. pylori* in 48.2% of adenotonsillectomy tissues in adults, as detected by rapid urease (CLO) test in the area of Shiraz (south of Iran). *H. pylori* –DNA was detected in resected adenoid tissue from 15% (8/53) of children (mean age=7.5 year) undergoing adenoid surgery in our center in Tehran (Noorbakhsh et al. (2011)

The etiology and microbial flora of nasal polyps in Iran is not well understood. This case control study was conducted in order to investigate *H. pylori* serology and to determine the presence of *H. pylori* -DNA in resected nasal polyp tissues.

METHOD AND MATERIALS

A case control study performed in the Department of Otorhinolaryngology, Rasul Hospital, Tehran, Iran between 2007 and 2008. The study was approved by the Ethical Committee in Research Center for Diseases of Ear, Nose, Throat in Iran University of Medical Sciences.

Cases included 51 adult cases (>12 years) undergoing nasal polyp surgery and 25 control adult cases undergoing elective repair surgery for nasal fracture.

Initially, a questionnaire was completed by an authorized physician, followed by a complete clinical examination.

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

Blood samples were taken for routine blood tests as well as serologic tests before surgery.

We excluded all cases with immunodeficiency states, diabetes mellitus, renal failure, patients receiving antibiotic at least 2 weeks before surgery, cases with known malignancy or other diseases proven from pathologic studies.

Blood samples (2 ml) were centrifuged and transferred to our research laboratory. The serum was stored at -20°c until the serologic examination was performed.

Specific *H. pylori* antibodies (IgG and IgA) in all cases and controls were assessed by ELISA.. The commercial kits (Chemicon-Germany) were used and the results were interpreted as suggested by the manufacturer. Results were calculated quantitatively.

During surgery, 1 cm^3 of resected polyp tissue in polyposis cases and 1 cm^3 of inferior nasal turbinate mucosa in controls were resected and placed in sterile tubes. Samples were centrifuged and homogenized, then preserved at -80°C

A PCR template Purification Kit (Roche; Germany) used for all prepared tissue samples. Steps for DNA–extraction were carried out according to the directions from the manufacturer. The binding column tube was transferred to a new 1.5 ml tube. The integrity of DNA was assessed by gel electrophoresis (1% agarose).

H. pylori- DNA was evaluated qualitatively by specific PCR primer kits (QIAquickP® QIAGEN; Germany). Diagnostic kits included a ready to use PCR mix kit, positive and negative controls and other qualified reagents along with a protocol for detecting as low as 10 copies/ml of the *H. pylori* genome.

Statistical analysis

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

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

RESULTS

Demographic results

Ages varied between 12 to 63 years; mean 37.5±13.7 years; 63% (39) of cases were male and 37% (23) female. Controls were between 18 to 25 years of age; Mean 31±11.5 years. Male to female ratio was 2:1.

PCR results

Positive *H. pylori* –DNA in nasal polyp tissues was 32.3% (20/62); Positive *H. pylori* –DNA in polyp cases was significantly higher than in control nasal turbinate tissues 4% (1/25) [(p-value=0.01; OR=11.4] (Figure 1).

Serologic results

H. pylori- IgA positively was not significantly difference between cases and controls [14.5% (9/62) vs 4% (1/25) of controls] (p-value=0.27, OR=4.1) (Figure 2).

Previous immunity against *H. pylori* (IgG) was significantly higher in the patient group [71% (44/62) vs 32% (8/25)] (p-value=0.001; OR=5.2) as shown in Figure 3.

Poor agreement between *H. pylori* –DNA (PCR) and serum *H. pylori*- IgA antibody (actual agreement=78.2%; p-value=0.005; Kappa=0.27); and H. pylori - IgG antibody (actual agreement=60%; p-value=0.001; Kappa=0.27) were observed in the nasal polyposis cases.

DISCUSSION

We detected a higher rate of previous infection with

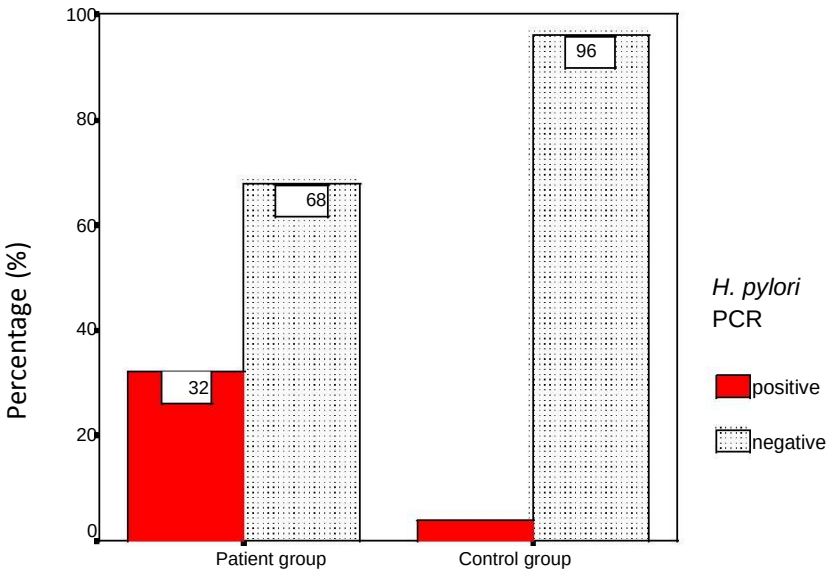

Figure 1. Distribution of *H. pylori* –DNA (PCR) in cases and controls.

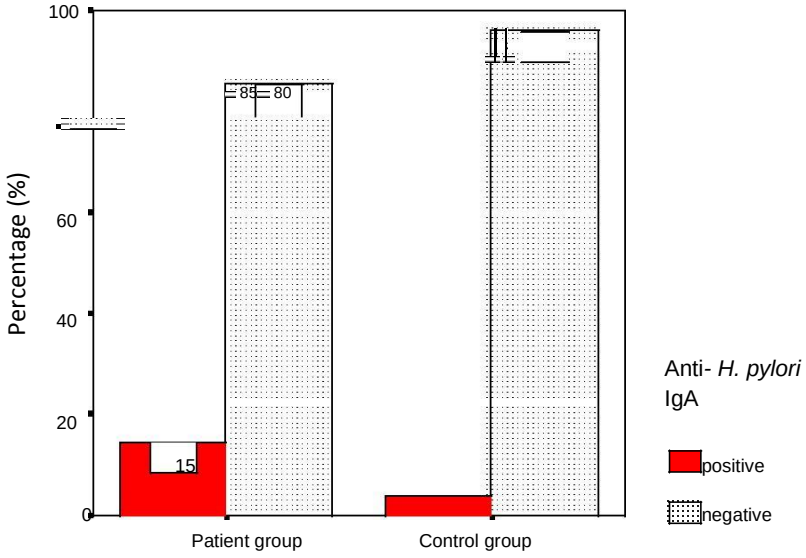

Figure 2. Distribution of *H. pylori* - IgA (ELISA) in cases and controls.

H. pylori in nasal polyposis cases by at least 2 specific diagnostic tests. Positive *H. pylori* –DNA was found significantly more often in polyp tissues compared to normal tissues from control subjects. Previous immunity against *H. pylori* (IgG antibody titers) was at least twice more common in polyposis cases. But this was not true for *H. pylori* -IgA antibodies between cases and controls (p-value=0.27).

Results of the present study are very similar to a previous Turkish study (Ozdek et al., 2003). In a Polish study, Szczygielsk et al. did not detect *H. pylori* infection by the ureae test in tissue, neither in cases nor in controls. Their poor results may be due to a less-sensitive method (Szczygielski et al., 2007).

These results suggest that chronic or persistant infection with *H. pylori* occured in polyp tissues of studied cases significantly more often than in healthy controls. However, serology consistent with acute *H. pylori* infection showed similar rates between cases and controls.

Results indicated that 70% of cases with nasal polyp had previous *H. pylori* infection but only 32.3% of infected cases would have chronic and persistant infection in nasal polyps (positive- DNA) for longer period.

We observed poor agreement between *H. pylori* –DNA

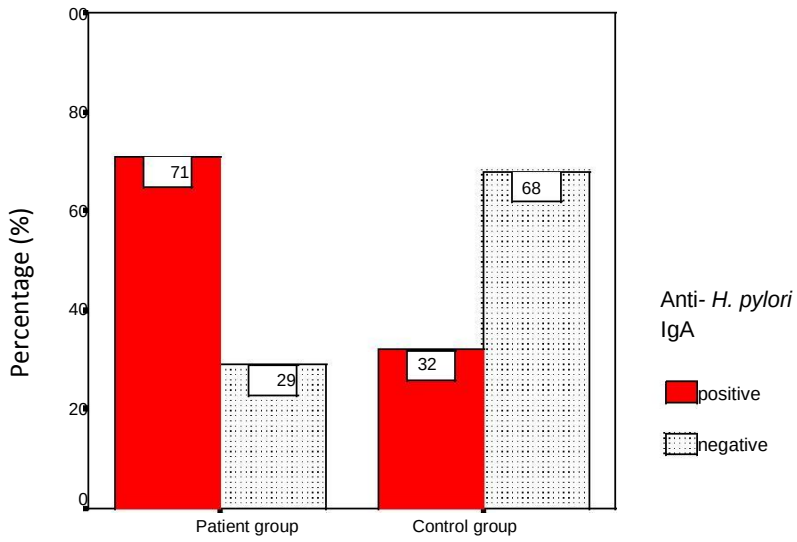

Figure 3. Distribution of *H. pylori* - IgG (ELISA) in cases and controls.

(PCR) in tissue and positive *H. pylori* –IgA and IgG antibody in serum. (Kappa index= 0.27). Serum *H. pylori* antibodies (ELISA) tests (IgA and IgM) in comparison with PCR has lower specificity for diagnosis of local infection in nasal polyp tissues. The positive serology could indicate colonization of the gastrointestinal tract or other sites including nasal polyps. The specific *H. pylori* culture or DNA of nasal polyp tissue in cases with positive *H. pylori* serology could differentiate active *H. pylori* associated with nasal polyps from simple colonization. Therefore serologic exams are not recommended for diagnosis of active infection in cases with nasal polyps. Indeed, false negative culture will occur if the cases received previous antibiotics (Cervin, 2001).

In our opinion, testing *H. pylori* -DNA in nasal polyp tissues is reliable and specific for diagnosis of active *H. pylori* infection. Previous antibiotic usage does not affect the PCR results.

Present results are similar to other studies (Dinis et al., 2006; Unver et al., 2001; Lukes et al., 2008; Khadem et al., 2005; Dagli et al., 2006; Skinner et al., 2001; Agirdir et al., 2006; Karlidag et al., 2005; Bulut et al., 2006; Szczygielski et al., 2007; Saffari et al., 2003; Masoodpoor et al., 2008; Baghaei et al., 2009; Nouraie et al.. 2009; Koc et al., 2004). Serologic results are very close to other studies done in Iran (Szczygielski et al., 2007; Saffari et al., 2003). 32% of our controls had previous immunity (IgG) against *H. pylori* infection.

Seroprevalence to *H. pylori* infection is high in the Iranian population (Szczygielski et al., 2007; Saffari et al., 2003). Initial infection probably occurs at an early age and its prevalence increases with age. The infection will increase to 30% in 2nd and 53.5% after the 4th decade of life (Szczygielski et al., 2007).

H. pylori –DNAs in cases with nasal polyps is very similar to the Khademi et al. (2005) study *H. pylori* infection has been found in tonsil and adenoid tissues of 48.2% studied cases (3 to 43 years) by the urease test, but were two times more frequent than in adenoid tissue (*H. pylori* –DNA) of studied children (with mean age 7.5 years) studied in our center (32.3 vs 15%).

Indeed positive *H. pylori*- IgA was reported in 15% and positive *H. pylori* – IgG in 11% of children with rhinosinusitis > 2 weeks (mean age 4.2 years). Although *H .pylori* infection varies between countries and often within a country an older age for cases in the Khademi et al. (2005) study is the probable cause for this higher infection compared with studied children Saffari et al studied *H. pylori* antibodies in a population in Shiraz (south of Iran) (Saffari et al., 2003) where 28.3% of persons between 20 to 40 years and 32% of the population between 41 to 80 years had positive *H. pylori*-IgG. Positive *H. pylor*i–IgA was also observed in16.7 and 53.5% respectively (Saffari et al., 2003)

We concluded that *H. pylori* infection is high in the Iranian population. Initial *H. pylori* infection might occur at an early age (4 years) in our country, the prevalence of *H. pylori* infection increasing with age. The infection increases to 30% in the 2th and to 53.5% after the 4th decade of life. Chronic and persistent infection (positive-DNA) is found in parts of the upper respiratory tract (nasal polyp adenoid hypertrophy) for longer periods. *H. pylori* infection was detectable in 15% of adenoid tissues in children before 8 years of age; adenoid tissues of 48% adult cases and in nasal polyp tissues of 32.3% studied persons in Iran.

The role of *H. pylori* infection in chronic rhinosinusitis has been elucidated in several studies (Cervin, 2001; Kurtaran et al., 2008; Morinaka et al., 2003; Dinis et al., 2005).

Results of the Koc et al. (2004) study are very similar to ours. They found specific IgG antibodies to *H. pylori* in 86.7% (26/ 30) polyp patients and 85% (1/ 20) controls. *H. pylori* were identified in the nasal polyp tissue of 20% (6/ 30) of patients but not in controls. No significant statistical difference was observed for *H. pylori* antibodies (ELISA) among the cases with nasal polyps and the controls (P = .59) but a statistical difference was seen between 2 groups by immunohistochemical staining in tissue, P = 0.037.

Recent study by Ozyrut et al. (2009) determined real-time PCR assay (RT PCR) as more sensitive in detecting *H. Pylori*.

The possible role of *H. pylori* as a trigger for some extragastric diseases had been largely investigated in the last years. Several studies concerning cardiovascular diseases, neurological disorders, diabetes mellitus, ear and eyes diseases, immunological and hematological disorders, liver and bile tract diseases, gynecological and respiratory tract pathologies (Figura (year???)). The antibiotics administration to reduce the inflammation and virulence of infection may be useful in reducing tissue damage in chronic rhinosinusitis cases with nasal polyp (Cervin, 2001).

As a result, the administration of antibiotics to reduce inflammation and virulence of infection may be useful in reducing tissue damage in chronic rhinosinusitis with nasal polyps (Cervin, 2001).

Treatment for longer period with suitable antibiotics may eradicate *H. pylori* infection and reduce the size of nasal polyps.

The data presented here is compatible with *H. pylori* having a possible role in cases with nasal polyps but association does not prove causation.

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

Limitations of the study

The results define a possible role for *H. pylori* infection in nasal polyps but association does not prove causation. More studies are needed to evaluate the role of *H pylori* in the etiology of nasal polyps. The use of more specific methods such as real time- PCR; or specific culture may further elucidate the role of *H. pylori* infection in nasal polyps.

Conclusion

We define a possible role for *H. pylori* infection in nasal polyps and recommend the PCR as the best method for *H. pylori* infection. Whether or not *H. pylori* play a pathogenic role cannot be determined from the data obtained in this investigation. More studies are needed to evaluate this correlation.

If such causation is established then long-term antibiotics treatment in cases with nasal polyps that do not respond to surgery or steroids may be useful.

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