The significance of Helicobacter pylori in patients with nasal polyposis

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ABSTRACT

Aim To determine the presence of Helicobacter pylori in nasal polyps and the transmission of H. pylori from stomach to nasal polyps.

Methods In a prospective, controlled clinical research, 35 subjects with nasal polyps and 30 controls with concha bullosa (CB) were involved, and had been subjected to endoscopic sinus surgery (ESS). In the biopsies of removed polyps and CB, polymerase chain reaction (PCR) was used for detecting the H. pylori DNA. Blood samples of the test and control group were evaluated for H. pylori immunoglobulin (Ig) G and A antibodies by ELISA. The test and control group underwent esophagogastroduodenoscopy with taking biopsies from the stomach for PCR detection of H. pylori DNA.

Results In blood samples, specific IgG and IgA antibodies to H. pylori were found in 30 (85.71%) of 35 polyp patients and in 16 (53.33%) of 30 controls. In 10 (28.57%) of 35 patients H. pylori DNA was identified in the nasal polyp tissue, but it was not detected in the CB specimens. H. pylori DNA was found in the stomach mucosa samples of all test and control group of subjects. Significant statistical difference was found in the H.pylori specific IgG and IgA between the test and control group (p<0.006) and between the polyp and control biopsy specimens of H. pylori DNA by PCR (p<0.001).

Conclusion This research points to the colonisation of nasal polyps with H. pylori, the transfer of H. pylori from stomach to nasal polyps and potential role of H. pylori in emergence of nasal polyps.

Key words: H. pylori, nasal polypus, PCR, ELISA
INTRODUCTION

The history of nasal polyposis reaches far back to ancient past, since writings concerning this disease appear in 4,000 years old papyruses. Despite being known for such a long time, it remains a great mystery even today and the exact cause of the disease is unknown (1). Mechanisms assumed to have some influence in the emergence of nasal polyps are allergy, infection, autonomic imbalance, mucopolysaccharidosis, enzymatic disorder, sensitivity to medication, mechanical obstruction, histamine, proto-oncogenes and, more recently, gastroesophageal reflux disease (2). Recent insights concerning a high prevalence of Helicobacter pylori infection among patients suffering from various other diseases outside of the digestive system, e.g. among patients with acute coronary diseases, some skin diseases (chronic urticaria, rosacea), immune and vascular disorders etc., point to its potential role in the aetiologies of these conditions (3). In the last fifteen years, the number of studies indicating the relation between chronic rhinosinusitis and gastroesophageal reflux is increasing, from proving the presence of H. pylori on aspirates and sinus biopsies to pH-metric evidence of increased acidity and pepsin in epipharynx and nasal cavities in some patients with gastroesophageal reflux (4,5). These studies are particularly related to paediatric population, where a significant improvement in the rhinosinusitis symptoms has been evidenced following treatment with proton pump inhibitors (6,7,8,9,10). H. pylori is a slow pathogenicity bacterium that actively alters the immune response of an infected host, constantly supporting the imbalanced state between the bacterium’s multiplication and the host’s immune response (11). This explains the existence of a long-term (chronic) inflammatory reaction of relatively low intensity, which is characteristic in the pathological signs of H. pylori caused chronic gastritis (12). H. pylori organisms have been found in the sinonasal tissue by applying different diagnostic methods (13,14). Further, the mode of H. pylori infection spread to nose and sinuses has not been fully clarified yet. According to present knowledge, the basic route is human-to-human. It is assumed there are two modes of transmission: oral-oral (or stomach-to-mouth) and faecal-oral (15,16).

Main goal of our study is to determine the presence of H. pylori in nasal polyps and to determine the transmission of H. pylori from stomach to nasal polyps. To detect H. pylori we used PCR detection of H. pylori DNA biopsy specimens and enzyme-linked immunoadsorbent assay (ELISA) of sera.

PATIENTS AND METHODS

In the prospective clinical research, 35 patients with nasal polyps and 30 controls with bullous middle nasal concha (CB) were involved and had been subjected to endoscopic sinus surgery (ESS) at the Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Centre of Osijek, Croatia.

Patients were included in the test group if they had histologically diagnosed nasal polyposis. Patients with CB without any signs of nasal polyposis constituted the control group. If they had any nasal pathology beyond CB on endoscopic or computed tomography (CT) exam they were excluded from the study.

Subjects with the following conditions were excluded from the research: ulcer disease, chronic atrophic gastritis with a history of taking H2 blockers, antacids or proton pump inhibitors one week prior to the surgical procedures or antibiotics within four weeks.

Prior to the ESS, serums of both groups were analyzed for H. pylori specific immunoglobulins (IgG, IgA) by ELISA. All subjects had undergone esophagogastroduodenoscopy prior to the ESS and two stomach biopsies were taken from antrum and corpus. The biopsies were frozen at -80°C for PCR detection of H. pylori DNA. Each ESS was done with local intensified anesthesia. Multiple biopsies were taken from the patients with nasal polyposis and from patients with CB. The biopsies were frozen at -80°C for PCR detection of H. pylori DNA. If two of the specimens from a patient were confirmed to be positive, that patient was recorded as positive for H. pylori (or when PCR was positive).

The pathologist was unaware of the ELISA and PCR test results and the clinical diagnosis. All specimens were examined by one experienced pathologist from the Department of Pathology University Hospital Centre Osijek, Croatia. The study design was approved by ethics committees of the Clinical Hospital Center of Osijek and School of Medicine of the Josip Juraj Strossmayer University in Osijek.

All patients gave their written informed consents before being included in this study.
**Serologic Analysis**

Sampled vein blood was centrifuged at 2500 rpm for 5 minutes. Using the enzymatic immunoassay DRG *Helicobacter pylori* IgG, IgA ELISA kit (DRG Instruments, Marburg, Germany) on a microtiter plate, IgG and IgA antibodies for *Helicobacter pylori* in human serum were determined. Microtiter wells as a solid phase are coated with recombinant *Helicobacter pylori* Cag A antigen. Diluted patient specimens and ready-for-use controls are pipetted into these wells. During incubation *Helicobacter pylori*-specific antibodies of positive specimens and controls are bound to the immobilized antigens. After a washing step to remove unbound sample and control material horseradish peroxidase conjugated antihuman IgG, IgA antibodies are dispensed into the wells. During the second incubation this anti-IgG, IgA conjugate binds specifically to IgG, IgA antibodies resulting in the formation of enzyme-linked immune complexes. After the second washing step to remove unbound conjugate, the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and the development of blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid. The intensity of this color is directly proportional to the amount of *Helicobacter pylori*-specific IgG, IgA antibodies in the patient specimen. Absorbance at 450 nm is read using an ELISA microtiter plate reader. The sensitivity and specificity of this kit are 99% and 97%, respectively (17).

**PCR Analysis**

DNA was isolated from the nasal polyp tissue samples, mucosa of bullous middle nasal concha and mucosa of stomach antrum and corpus using the QIAGEN DNaseasy tissue kit mini-spin columns according to the manufacturer’s instructions (QIAGEN, Hilden, Germany). Obtained tissue samples of 25 mg were placed in a 1.5 mL microcentrifuge tubes, added 180 uL buffer ATL, and 20 uL proteinase K, and incubate at 55 °C in a heat block until tissue is lysed. It was shaken occasionally, every 20-30 minutes. The incubation take approximetly 1.5 hours. After 1.5 hours it was added 200 uL buffer AL to sample, mixed by hand, and incubated at 70° C for 10 min. in a heat block. Than 200 uL ethanol (96-100%) was added to the sample, mixed thoroughly by hand. DNaseasy mini column was placed in a 2 mL collection tube, the mixture was pipetted from the microcentrifuge tube into the mini-column so that the mixture touched the filter but the pipette tip did not, it was centrifuged for 1 min. at maximum speed (8000 rpm). Mini-column was placed in a new 2 mL collection tube, discarded flow-through, 500 uL buffer AW1 was added and centrifuged for 1 min. at maximum speed. Then, mini-column was placed in a new 2 mL collection tube, discarded flow-through, 500 uL buffer AW2 added and centrifuged for 3 min. at maximum speed. The filter was spun dry so there was no ethanol. After that the mini-column was placed in a new 1.5 mL microcentrifuge tube, pipetted 100 uL buffer AE directly onto the mini-column membrane, incubated at room temperature for 1 min., and then centrifuged for 1 min. at 8000 rpm. Leachates potentially containing *Helicobacter* DNA were analyzed using the standard fluorescent ABI Helicobacter plus – minus PCR assay, and the final fluorescence values were read using the ABI Prism 7000 real-time PCR device and analyzed with the appertaining software (18).

**Statistical Analysis**

The Pearson chi-square test was used to examine the relationship between the test and control groups. A P value less than 0.05 was considered significant. Statistical analyses were performed with SPSS for Windows, version 15.0., Chicago, IL, USA.

**RESULTS**

The nasal polyposis group comprised 35 consecutive patients: 25 (71.43%) males and 10 (28.57%) females. The median age of the patients was 54 years, with a range between 27 and 78 years, whereby the highest number of the patients was in the age group between 53 and 65 years (n=13, 37.14%) (Figure 1). The control group with CB

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**Figure 1. Distribution of patients with nasal polyposis according to age and gender**
consisted of 30 patients, of whom 12 (40%) were males and 18 (60%) were females, ranging in the age from 19 to 75 (median age 42.5) years, while the highest number of patients was in the age group of 33 to 46 (n=18, 60%) (Figure 2).

*H. pylori* specific IgG and IgA antibodies were detected in 30 (85.71%) of 35 patients with polyp, and in 16 (53.33%) of 30 controls. Negative results for the presence of IgG and IgA antibodies were found only in 5 (14.29%) out of 35 patients with nasal polyposis and in 14 (46.67%) out of 30 control group subjects (Table 1).

Ten of 35 (28.57%) biopsy specimens of polyps were positive, whereas none of the control group samples were positive for *H. pylori* DNA in PCR analysis. The patients with polyp in which *H. pylori* DNA was detected by PCR had *H. pylori*-specific IgG and IgA in the serum.

*H. pylori* DNA was detected in the stomach mucosa samples of all test and control group of subjects by PCR analysis.

Twenty six (out 74.29%) patients with nasal polyps and 18 (out 60%) patients with CB in the stomach mucosa samples were shown to be positive for *H. pylori* by histology (Table 2) (p=0.224).

Significant statistical difference was found in the *H. pylori* specific IgG and IgA between the test and control groups (p<0.006). The comparisons between the polyp biopsy specimens and the control biopsy specimens by the PCR analysis of *H. pylori* DNA revealed a significant statistical difference (p<0.001).

**DISCUSSION**

Nasal polyposis is marked by the formation of benign, stem-like growths on the mucous membrane with 1-4% prevalence in the total population and growing tendency (2). It is more frequent in males and its incidence increases with age (2), as confirmed by this research. It is considered today that nasal polyps form as a result of local reaction of the sinus mucous membrane with a resulting mucosal hyperplasia as the secondary response to chronic inflammation (2). However, the initial or persisting stimulus for the chronic inflammation remains unknown (2). Recently, it has been considered that gastroesophageal reflux is in connection with several aerodigestive disorders, especially with sinusitis (19). Although the mechanism by which the gastroesophageal reflux causes sinusitis is unclear, we know that exposure to acid leads to mucosal oedema, secretion and chronic inflammation of the respiratory mucosa (19).

*H. pylori* colonizes the stomach, which is the most suitable medium for its survival. This is a medium with acid pH level, low oxygen concentration and all the ingredients that the microorganism requires, i.e. an environment where no other infective agent can compete with the bacterium. However, *H. pylori* was also discovered in other areas adjacent to the stomach, such as esophagus, duodenum, feces, oral cavity, saliva, dental plaque, tonsils and even nasal cavity (20,21).

There are contradictory data among studies about the presence and possible role of *H. pylori* in emergence of nasal polyps (13,14,20,22-26). Some studies (13,20,22,23) have confirmed the colonisation of *H. pylori* in nasal polyps and possible impact on the pathophysiology and management of nasal polyps. On the other hand, some studies (14, 24-26) did not find *H. pylori* in the tissue specimens of patients suffering from nasal polyposis. Furthermore, there are large differences in sensitivity and specificity of the diagnostic methods used in previous studies for the detection of *H. pylori* in the...
nasal polyps (26). In the previous studies (13,14) researchers did not use highly sensitive and gold standard methods for H. pylori detection, such as PCR, so the negative and positive results could be criticized (26). On the other hand, in recent studies there are contradictions among researchers who used PCR for detecting H. pylori DNA in nasal polyps and control tissues (23,26). Ozyurt et al (23) detected H. pylori DNA by real-time PCR in nasal polyps, but also in normal nasal mucosa samples, and in larynx samples. However, Nemati et al (26) used three methods (i.e. PCR, culture, and urease test) to detect H. pylori and all three tests in the biopsied specimens of nasal polyps and in the control group were negative for H. pylori. They concluded that H. pylori can be considered as an accidental finding, rather than an etiological factor, in patients with nasal polyposis (26). However, they based their conclusions on patients without gastroesophageal reflux signs and symptoms and without investigating H. pylori DNA in stomach samples (26). So, they did not reveal the mechanism by which H. pylori colonizes nasal cavity or not. That mechanism is still unknown, and could be explained by three possibilities (13). First, the nasal cavity can be H. pylori reservoir (23). Second, the oral cavity can represent H. pylori reservoir and microorganisms can reach the sinonasal cavity by oronasal reflux (27). Third, stomach can be the primary infection reservoir and the transmission of H. pylori from stomach to nasal cavity can occur by means of gastroesophageal reflux (13).

All the aforementioned studies did not analyse nasal polyps and stomach mucosa for H. pylori DNA in regard to revealing the colonisation and transmission of H. pylori into nasal cavity. This study, for the first time ever, used real-time PCR, a gold standard method for H. pylori detection, to detect H. pylori DNA in tissue samples of nasal polyps, CB and stomach mucosa. The results of this study have shown the presence of H. pylori DNA in stomach mucosa of all tested and control subjects, in nasal polyp tissues (28.57%), but not in CB tissues.

It can be concluded that stomach is the primary H. pylori reservoir, it is transferred to the nasal cavity by gastroesophageal reflux, and consequently it colonizes nasal polyps, as opposed to the control subjects with CB where no H. pylori was found. The results of this study have shown that real-time PCR is an appropriate method to assess H. pylori in tissues due to its sensitivity and specificity. Other methods assessed in this study (i.e., histology, serology) produce many false-negatives.

It should be emphasized that further epidemiological studies are necessary to use real-time PCR in order to prove the presence of H. pylori DNA in nasal polyps and stomach mucosa as a potential pathogenetic mechanism in the development of nasal polyps.

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TRANSPARENCY DECLARATIONS
Competing interests: none to declare.

REFERENCES
Značaj Helicobacter pylori u bolesnika s nosnom polipozom

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SAŽETAK

Cilj Utvrditi prisutnost Helicobacter pylori u nosnim polipima i utvrditi prijenos H. pylori iz želuca u nosne polipe.

Metode U prospektivno, kontrolirano kliničko istraživanje, uključeno je 35 ispitanika s nosnom polipozom i 30 kontrolnih ispitanika. U uzorcima krvi, PCR-om je utvrđivana prisutnost Helicobacter pylori DNA. U uzorcima sluznice želuca, PCR-om je utvrđivana prisutnost helicobacter pylori DNA između bioptičkih uzoraka polipa i kontrolnih uzoraka polipa.

Rezultati Uzorci krvi, H. pylori specifična protutijela klase IgG i IgA, pronađeni su u 30 (85,71%) od 35 pacijenata s polipima, i u 16 (53,33%) od 30 kontrolnih ispitanika. U 10 (28,57%) od 35 pacijenata H. pylori DNA je otkrivena u tkivu nosnih polipa, ali nije otkrivena u uzorcima srednje nosne školjke. H. pylori DNA otkrivena je u uzorcima sluznice želuca u svih ispitanika, te otkrivena u 30 (85,71%) od 35 pacijenata s polipima.

Zaključak Ovo istraživanje ukazuje na kolonizaciju nosnih polipa s H. pylori, na put prijenosa H. pylori iz želuca u nosne polipe, te na moguću ulogu H. pylori u nastanku nosnih polipa.