

Investigation of association of *Helicobacter pylori* and simple nasal polyps

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Abstract

Objective: To investigate a possible contribution of *Helicobacter pylori* (H. Pylori) in the etiopathogenesis of simple nasal polyps.

Study Design: Prospective clinical trial.

Methods: Twenty five patients with simple nasal polyps underwent nasal polypectomy were studied. *Helicobacter pylori* DNA was investigated for specimens removed from those patients by using polymerase chain reaction (PCR).

Results: *Helicobacter pylori* DNA was detected in 2 (8%) of total 25 specimens.

Conclusion: We could not find strong indicator that H. pylori played a role at the tissue level in the pathogenesis of nasal polyp.

Keywords: Nasal polyp - *Helicobacter pylori* –Polymerase chain reaction.

Helicobacter pylori has been investigated in several other organ systems and localizations such as the oral cavity, but has not been investigated in simple nasal polyps a region that could be indirectly exposed to the bacterium.

Nasal polyps are common in patients with chronic rhinosinusitis, yet, to date, theories only are known about the pathophysiology of nasal polyp formation. It does appear to be partially related to the patient's underlying allergic status. However, nasal polyps may occur even in the absence of detectable environmental allergies¹. A myriad of inflammatory mediators have been implicated in their pathogenesis². In addition, for reasons that remain unclear, the natural course of nasal polyps is unpredictable.

H. pylori are a microaerophilic, gram-negative spiral organism that can cause gastric and duodenal ulcers, or even gastric cancer³⁻⁵. Although H. pylori infection is widespread throughout the world, the exact mode of transmission has not yet been fully elucidated⁶.

Possible modes of transmission are through the oral-oral, fecal-oral, and gastrointestinal-oral routes⁷. Human stomach was considered to be the only reservoir of H. pylori until the bacteria were discovered in human dental plaque, oral lesions, and saliva⁸⁻¹⁰. Recently, this organism was also detected in tonsil and adenoid tissue after adenotonsillectomy, as well as in mucosa of patients with chronic rhinosinusitis¹¹⁻¹³. Thus, contribution of H. pylori to the etiopathogenesis of simple nasal polyp is possible. The present study was planned to investigate the presence of H. pylori by polymerase chain reaction (PCR) in nasal polyp specimen.

A variety of diagnostic techniques for the detection and identification of bacteria in clinical samples, based on characteristic DNA sequences, have been documented. Nucleic acid-based detection systems can allow the identification of bacteria without the need for isolation in pure culture or the propagation of living organisms¹⁴. This technique is especially useful for the detection of organisms that cannot easily be grown in vitro¹⁵, as is the case for H pylori¹⁶. PCR, a technique for the amplification of DNA sequences in vitro, has been widely used to assist in the diagnosis of infectious diseases (17-19). The speed and sensitivity of the technique make it ideal for "high-throughput"

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automated screening of blood and tissue samples. Furthermore, PCR can detect a single copy of a target DNA sequence and therefore requires only small samples for analysis.

Material and methods

Materials

In this study, polymerase chain reaction was performed to detect H. pylori DNA for 25 specimens from patients with simple nasal polyps who underwent nasal polypectomy between January 2005 and December 2007 in Basrah General Hospital, while polymerase chain reaction study performed in Basrah College of sciences. There were 17 men and 8 women, aged between 21 to 65 years (mean: 38.4 years).

Patients were subjected to an assessment protocol that included careful history review; full ear, nose, and throat examination, emphasizing on laryngeal examination by recordable telescopic examination to visualize changes in posterior aspect of larynx (edema and congestions which indicates posterior laryngitis). All patients were questioned about the classic symptoms of gastroesophageal reflux (heartburn, acid taste, and regurgitation).

Methods

Simple nasal polyps of 25 patients were removed by the conventional method of intranasal polypectomy, and then biopsy specimens were immediately frozen and stored at -20°C until used. For DNA extraction, tissue samples were manually homogenized in 0.5 mL of Tris-EDTA-sodium chloride lysis buffer (10-mmol/L Tris hydrochloride [pH 8.0], 1-mmol/L EDTA, 100-mmol/L sodium chloride [pH 8.0]) per 100 mg of tissue. Proteinase K was added at a final concentration of 100 $\mu\text{g}/\text{mL}$. The mixture was incubated at 56°C for 3 hours before the enzyme was inactivated by heating the sample for 10 minutes at 95°C . The mixture was centrifuged (13 000g, 1 minute) and the supernatant retained, and genomic DNA was purified by the phenol-chloroform method (20). The DNA was then precipitated with ethanol, pelleted (13 000g, 5 minutes),

washed in 70% ethanol, and dried. The dried pellet was re-suspended in 20 μL of sterile water and stored at 4°C .

H pylori PCR primers and PCR amplification

The primers selected for PCR targeted the 16S ribosomal RNA (rRNA) gene of H pylori. These primers were previously described and tested in other studies, in which their specificity for H pylori was confirmed^{21,22}. The primers used were H pylori forward primer (5'-CGTTAGCTGCATTACTGGAGA-3') and H pylori reverse primer (5'-GAGCGCGTAGGCGGGATAGTC-3') (Genosys, Cambridgeshire, England). The expected size of the amplified product was 295 base pairs (bp). Before PCR amplification and to avoid DNA contamination as well as cross-contamination, all pipettes, tubes, and racks were exposed to UV light for 20 minutes before setup in a cabinet using filtered air. The reaction components were assembled on ice in sterile 0.5-mL thin-walled Eppendorf tubes and mixed by vortexing. The PCR amplification was carried out in a total volume of 50 μL comprising 1.0 U of Taq polymerase in reaction buffer (Boehringer Mannheim Biochemica, Mannheim, Germany), 1.5-mmol/L magnesium chloride, 30 pmol of each primer, 0.2-mmol/L of each deoxyribonucleotide triphosphate, and 5 μL of extracted tissue DNA. The reaction mixtures were overlaid with mineral oil (Sigma-Aldrich Corp, St Louis, Mo), incubated at 96°C for 2 minutes, then subjected to 40 cycles of 96°C for 60 seconds, 60°C for 60 seconds, and 72°C for 90 seconds, followed by a 10-minute extension at 72°C . In addition, each set of reactions incorporated a positive control, DNA extracted from an H pylori isolate, and a negative control with the DNA template replaced with double-distilled water. Amplification products were analyzed, after electrophoresis at 80 V, in 1.5% (wt/vol) agarose gels stained with ethidium bromide; along with a 100-bp DNA ladder (Promega, Madison, Wis) as a size marker.

Results

The present study includes 25 patients with simple nasal polyps, 17 were males and 8 were females, their ages ranged between 21 and 65 years (mean 38.4 years).

Thirteen patients out of the total 25(52%) gave positive history of gastroesophageal reflux symptoms (heartburn, acid taste, and regurgitation), but only 4 patients(16%) had positive laryngoscopic findings (posterior laryngitis),and interestingly H. pylori was observed in specimens of 2 patients (8%),both of them belong to the group(4 patients)who had positive laryngoscopic findings of GER.

Discussion

This study performed to detect H. pylori in tissues of simple nasal polyps. Because literature^{12, 13} had mentioned that H. pylori were isolated from the mucosa of patients with chronic rhinosinusitis, we thought that we can detect the organism in polyp tissue.

The application of PCR with respect to H. pylori is useful for detection purposes. Nested PCR was used for the detection of H. pylori in specimens from several sites in different regions in the body. The sensitivity and specificity of PCR are important issues, but primers used in the present study have been validated previously and used in other studies^{23,24}.

The passage of nasopharyngeal content into the nose is probably a common bad habit in patients with nasal obstruction to clean their noses. The negative pressure produced in the nasal cavity may cause reflux of the nasopharyngeal content into nose. In GER, gastroesophagonsopharyngeal reflux has been shown with pH monitoring studies²⁵⁻²⁹. Thus, in this study, the possible role of H. pylori in simple nasal polyp was investigated. No data supporting such a relationship could be found.

In recent studies, a close relationship between GER and chronic sinusitis was detected^{12, 13}. Ozdek et al.³⁰ reported a possible role of H. pylori in chronic rhinosinusitis and found H. pylori positive in 4 of their 12 patients with chronic rhinosinusitis. In another study, Unver et al.¹¹

investigated H. pylori colonization in adenotonsillectomy specimens and found a 57.89% rate of the presence of H. pylori. Skinner et al³¹. found 28% of their patients seropositive for H. pylori. Until now, H. pylori colonization in simple nasal polyp has not been investigated. In the present study, H. pylori was detected in 2 (7.9%) of 25 patients with simple nasal polyp. To the best of our knowledge, there has not been a study in the published literature so far investigating the existence of H. pylori in simple nasal polyp tissue. We also did determine the presence of H. pylori in two specimens. The failure in the present study to detect H. pylori in large number of specimens would tend to suggest that the nose and paranasal sinuses are not a permanent reservoir of H. pylori. Furthermore, the data presented in the current report strongly support the clinical impression that H. pylori are not the offending organism in the pathogenesis of simple nasal polyp. Therefore, we think that an ascending route for H. pylori infection in simple nasal polyp is unlikely.

Conclusion:

We could not find significant indicator that H. pylori played a role at the tissue level in the pathogenesis of nasal polyp, but these results should be confirmed by broad study including larger numbers of patients and different methods of detection of H. pylori.

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