Original Article

Investigation of Helicobacter Pylori in the mucosa of sinuses of patients with chronic sinusitis

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ABSTRACT

Objective: To investigate the presence of *Helicobacter pylori* in sinonasal mucosa of patients with chronic sinusitis

Design: A prospective case-control study

Materials and methods: Mucosal specimens were collected from the mid-third middle meatus and lateral side of mid-cornea. *H. pylori* has been investigated using PCR after DNA extraction and urease test.

Results: *H. pylori* was not found in any of the sample taken from both groups (case and control patients).

Conclusion: This is the first reported study to investigate the presence of *H. pylori* in sinonasal mucosa in Iran. In this study, *H. pylori* was not determined in these sites, although its possible presence could not be excluded. Thus, further investigation on more patients and application of sensitive diagnostic techniques are recommended.

Key Words: Helicobacter Pylori, Chronic Sinusitis, PCR, Urease Test

Introduction

Helicobacter pylori (HP) is a gram negative, microaerophilic, spiral organism which has been shown to be a causative factor in large cases of gastritis, peptic ulcer and it is also associated with gastric cancer (1,2,3). The common site of this bacterium is the human gastric mucosa (4). Despite of prevalence of the HP infection throughout the world, the precise route of transmission has not yet been fully realized. Because of vicinity of oral cavity, nose and sinuses to the digestive system, direct transmission of HP infection from to these sites are quite possible. Using different diagnostic procedures, presence of HP in specimens of dental plaques, saliva, tonsils, adenoids and oral lesions has been reported in some studies (5,6,7). If it can be demonstrated that HP may be capable of colonizing in other organs and tissues, the colonization can act as a reservoir of

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HP and may have an efficient role in recurrent infections. Therefore, recognizing the possible site of colonization outside the stomach can help the eradication of HP infection and prevent any recurrence.

Recently, the role of gastroesophageal and esophagonasopharyngeal refluxes in pathogenesis of chronic sinusitis has been highly considered (8,9). It has been assumed that inflammation and edema induced in nasopharynx are related with the reflux of gastric content and its exposure with nasopharyngral area, which can result in blockade of sinus opening and subsequent chronic sinusitis (8). However, the role of gastroesophageal reflux in inducing sinusitis remains a controversial issue. Moreover, according to the results of some studies, it is likely that other mechanisms except direct exposure of nasal mucosa to gastric secretion, has a potential role in the pathogenesis of chronic gastritis(10). Of course, certain mechanism of this phenomenon has yet to be determined (8).

Because the role of gastroesophageal reflux in the pathogenesis of chronic sinusitis has been doubted(10) and there is a direct relationship between HP colonization in the upper GI and the gastroesophageal reflux, this aim of this study was to investigate the presence of HP in sinonasal mucosa of patients with chronic sinusitis by using the urease test and polymerase chain reaction (PCR) (11,12). In addition, it seems that no research or investigation has yet been carried out in our country.

Materials and Methods

Patient Selection

This study is an applied and case-control study which has been performed on 44 patients in ENT clinic of Shahid Mostafa Khomeini Hospital in Tehran from May 2004 until August 2005. The case group in this study includes 22 patients with chronic sinusitis (confirmed by coronal CT scan of paranasal sinusitis). There are 6 patients with gastroesophageal reflux with positive HP in gastric biopsy. Due to lack of therapeutic response, all of these patients required an endoscopic surgery of the sinus. The control group in this study includes 22 patients with deviated nasal septum but without any sign of sinusitis (in coronal CT scan from paranasal sinuses). Because of the deviation of nasal septum, all of these patients have undergone a surgical operation.

Tissue Specimens

All specimens were taken aseptically from the mid-third middle meatus (by the uncinate process) and lateral side of mid-cornea (by sterile Beleckesly). Because HP grows diffusely in the mucus, a sample was taken from each side of nasal cavity of the patients. Samples 1 and 3 (by the uncinate process) were placed in urease container and samples 2 and 4 ((by sterile Beleckesly)) were located in PBS container.

After specimen collection, PCR test was carried out in the laboratory of Microbiology (Shahed University, Tehran, Iran). During transportation of samples, cold chain was protected.

DNA Extraction

DNA extraction was carried out by DNA-TM kit (Cinnagen, Iran, Cat No. DN8115c). Briefly, 25-50mg of specimens was added to 5µl protease and 100µl protease buffer. After incubation (in 55ºC for 2 hours) of these treated sample tissues, they were mixed with 400µl lysis solution and 300µl precipitation solution. Tubes were placed at -20 ºC for 20min and then centrifuged at12000g for 10min. after decanting, tubes were washed twice with 1ml wash buffer and pellets were dried at 65ºC and then were suspended in 50µl of solvent buffer. After centrifugation, the supernatant containing purified DNA was collected.

Polymerase Chain reaction (PCR)

PCR for detection of HP DNA was performed by using Helicobacter pylori PCR detection kit (cinnagen, Iran, Cat. No. PR 7843c). After defrosting the reagents, 0.2µl Taq-DNA polymerase and 20µl 1X PCR MIX were added to the tubes labeled for tests and positive and negative controls. Then 10µl purified DNA, or positive and negative reagents were added to the separate tubes and mixed for 3-5s. Tubes were transferred to thermocycler (Techne, UK) with

following program: 94C for 45s, 50ºC for 20s and 72ºC for 30s for 5 cycles and 94ºC for 20s and 50ºC for 20s and 72ºC for 30s for 30 cycles. At the end of program, 10µl of samples were electrophoresed by tris-acetate buffer in 1.5% agarose gel without adding the loading buffer. The presence of 492bp fragments indicated as positive results as shown in the positive control but not in the negative control. 100bp ladder was used for weight marker.

Urease Test

In urease test, samples were placed in urease medium and the released urease was observed in room temperature after 20min and thereafter at periods of 1, 3 and 24 hours. Discoloration from yellow to purple was indicated as a positive reaction.

Results

After data collection and completion of forms, data analysis was performed by SPSS software. In this study, 44 patients (24 male, 54.5% and 20 female, 45.5%) have been examined. The average of their ages was 32.8 ± 14.8 year. In the case group, all 22 patients were suffered from chronic sinusitis (15 males, 7 females with an average age of 39.8±17.1year). In the case group, there are 6 patients with reflux (confirmed by gastric endoscopy) and positive HP test. 19 patients in the case group have used different antibiotics, although these patients had not taken any antibiotic, H_2 antagonist and pump inhibitor since one month before the test. Three of six patients with reflux have used metronidazole and co-amoxiclav, and other three patients have used cefixime.

In control group, all patients had a deviated nasal septum (9 males and 13 females with an average age of 25.9±7.3 year), but none of them had chronic sinusitis and reflux.

The results of these tests in both case and control groups are negative and in PCR test, no band in 492 bp was observed which is indicative of absence of HP. In two cases, suspicious band adjacent to the reference point were seen, and for confidence, these specimens were again electrophoresed but all PCR result were negative.

Discussion

The possible role of the sinuses as a reservoir of HP is a controversial issue. This is the first study to investigate the presence of HP in sinonasal mucosa in Iran. Similar studies were reported by Morinaka (4) and Ozdeck.(13). Morinaka *et al.* studied 11 patients aged 20 to 72 years with chronic sinusitis by PCR, urease test and immunohistochemistry (IHC). Only three (16%) of 19 nasal and maxillary sinus specimens from two patients were shown to be HP positive (positive by PCR and IHC and weakly positive by urease test). In one of these two patients, HP infection of the stomach was confirmed (4). In the study of Ozdeck *et al.*, HP was detected in 4 of 12 patients with chronic rhinosinusitis by PCR but was not detected in healthy individuals (13). In addtion, three of four HP positive patients had gastroesophageal reflux-related complaints. Moreover, Ozdeck could not establish any relationship between HP and the development of chronic rhinosinusitis. In the present study, using PCR and urease test, we did not identify HP in sinonasal mucosa of 22 patients with and 22 patients without sinusitis. Existing of a very few number, abnormal form and patchy colonization of HP, using antibiotics and various sensitivities of detective tests are possible reasons for inconsistency of our results and that of the previous mentioned studies. The number of HP may have been under the lower limit of detection by PCR or urease test, so that Morinaka *et al.* have been reported that some negative specimens identified by these tests became positive by IHC (4). Moreover, it has been shown that in the presence of denaturated form of HP which is prevalent in clinical samples, the sensitivity of PCR and urease test and percentage of positive results are remarkably reduced (4). Because of the patchy colonization of HP, in this study, samples were taken from both sides of the nasal sinuses. Furthermore, since some drugs such as antibiotics, H2 antagonists and proton pump inhibitors can hinder the growth of HP, none of the cases in this study had taken these drugs since one month before the test (4). Prominent variation

in the sensitivity of diagnostic methods of HP infection has been demonstrated in other studies, especially in small numbers or in presence of denaturated forms of organism (4). In our study, PCR was selected, because it is established to be sensitive enough for gastric samples, although its sensitivity for sinonasal samples has not been evaluated. Sensitivity of urease test for detection of HP depends on the concentration of HP. For instance, in the presence of 10^6 bacteria per liter, the sensitivity of the test is 100%, whereas in 10^4/l and 10^3/l, urease test becomes 40% and 0% positive, respectively (4). Finally, the various prevalence of this bacterium in different populations was shown by Riggio (15), which leads in acquiring distinctive results in different studies.

Overall, in this study, we could not determine HP in the sinonasal mucosa of selected patients, although the possible presence of HP cannot be ruled out. Nevertheless, using more sensitive diagnostic techniques and further investigation on more patients can be recommended.

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