HELICOBACTER PYLORI IN NASAL POLYPOSIS

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ABSTRACT: Background: Nasal polyposis is an inflammatory condition of unknown etiology. Recently concern regarding GER or Helicobacter pylori as a possible pathologic cause of nasal polyps has been increasing. The present study was planned to investigate the presence of Helicobacter Pylor in Nassal polyps by PCR , rapid Urease test and serology. **Design:** Case control study. **Setting:** ENT ward of Shiraz, Khalilli Hospital, Iran. Period: April 2006 to March 2008. **Materials and Methods:** 37 patients with nasal polyps who had undergone nasal endoscopic sinus surgery and 38 control subjects who had undergone septoplasty and turbinectomy. Biopsy specimens of nasal polyps and inferior turbinates were assessed by PCR and Rapid Urease test. Blood sample of both study and control subjects were evaluated for anti H.pylori Ig G by ELISA. HP status was regarded as positive, if 2 tests were positive. **Results:** Seropositivity was more common in the patients with nasal polyps (72.97%) than in the control patients (31.57%) (P-value= 0.000) RUT was positive in 9 (24.3%) of 37 patients with nasal polyps, but was not positive in control group (P-value= 0.001). only 3 of (8.1%) of 37 patients with nasal polyps were positive for both RUT and ELISA (P-value =0.115). PCR was negative in all patients and controls. **Conclusions:** Polypoid tissue can be colonized by some other agents containing a urease enzyme other than Helicobacer Pylori. So, result of RUT can be false positive, and addition test may be performed. In the our study by using PCR , we were not able to confirm presence of Helicobacter pylori in the nasal polyps. However, further epidemiologic studies using different and specific diagnostic tests with control of documented GER is recommended.

Key words: Nasal polyps , Helicobacter Pylori , PCR , GER.

INTRODUCTION

Nasal polyposis (NP) is an inflammatory condition of unknown etiology. NPs are the most common benign intra nasal tumors[1,2]. They are present in approximately 2-4% of the population and can impair a person's quality of life[3,4]. Although NP has been recognized as an inflammatory process for many years, the true etiology of NP is mainly unknown[1,2,5].

Helicobacter pylori (HP) is a microaerophilic gram negative bacillus that has been shown to be the causative factor in a large portion of patients with Peptic Ulcer Disease (PUD) and gastric cancer. Human stomach was considered to be the only reservoir of HP, but recently this organism was also detected in oral lesions, dental plaque, saliva, tonsil and adenoid tissues[6,7,9].

Recently concern regarding gastroesophgeal reflux (GER) or HP as a possible pathologic cause of NP has been increasing[10,11,12]. It is assumed that transmission of HP may also occur through GER from the stomach to the nose and / or sinuses, which are located near the oral cavity, as well as through direct transmission from the mouth to the nose and/or sinuses. This knowledge brings the idea that there may be a relation between HP and the development of NP. However, the role of HP in the pathogenesis of NP has rarely been discussed.

The present study was planned to investigate the presence of HP by PCR, ELISA, and rapid urease test in patients with NP.

METHODS AND MATERIALS

This case – control study involved 37 adult patients with nasal polyposis who had undergone sinus endoscopic surgery, and 38 controls who had undergone septoplasty and turbinectomy at the ENT ward of Shiraz, Khalilli Hospital from April 2006 to March 2008.

Including Criteria

Patients were included in the study group, if they had histologically proven nasal polyps.

Excluding Criteria

Patients were excluded from study, if had history of H2 blocker and antiacid use in previous week, proton pump inhibitors use in last two weeks, or antibiotic use in last 4 weeks before operation. Patients were asked about previous surgical interventions, co-existing disease or allergic symptoms, smoking and medications. Also, patients were asked about classic symptoms of reflux (acid taste, regurgitation, heart burn).

Serologic Analysis

Serum samples obtained from all study groups were maintained at -20°C. Titers of anti H Pylori Ig G were assayed by using a serum enzyme linked immuno sorbant assay (ELISA) (IBL Company Kit). Any subject with a serum level reading of >10 U/ml was considered positive.

Tissue Analysis

Samples were collected either from the polypoid tissue that were obtained during endoscopic sinus surgery for Nasal Polypectomy or from mucosa of the removed parts of the inferior turbinate that were obtained during septoplasty and turbinectomy. Collected samples were immediately irrigated by saline and were partly cut out and 2x2 mm sized cut-out pieces were pushed beneath the surface of the Compylobacter-like organism (CLO) gel. After 0.5 hour and 24 hours we examined the colour change of the CLO kit. Changes from yellow to orange or red was recorded as positive. Other tissue pieces were immediately stored in -20°C until analyzed by PCR assay.

Polymerase chain reaction analysis

PCR was performed by using commercial Qinnagen Kit and analyzed according to the protocol of the company. After DNA was extracted from tissue specimen, 20 ml 1X PCR mix, 2 ml Taq DNA polymerase and 5 ml DNA were microfuged for 55 simultaneously thermal cycler program for PCR amplification was:

- First, 40 sec at 95°C, 20 sec at 50°C and 30 sec at 72°C for 5 cycles.
- Second, 20 sec at 94°C, 20 sec at 50°C and 30 sec at 72°C for 30 cycles.
- Electrophoresis of all of the PCR products was done using 1.5% agarose gel.

After the gels were stained with ethidium, the bands were examined by ultraviolet light. The gel electrophoresis of the 492 bp PCR product of H. Pylori was considered as positive.
(Figure1)

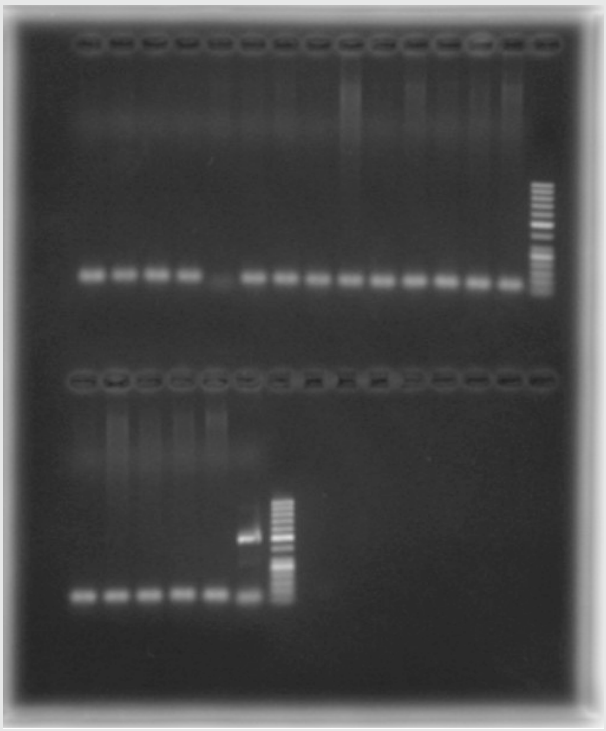

Fig-1. PCR for detection of H.Pylori

Radiologic evaluation

The severity of sinusitis was assessed by using Paranasal sinus (PNS) CT scan and according to the lund-mackey staying system (Table-I).

Table-I. Lund-mackay CT grading system

Sinus	Left	Right
Frontal	-	-
Anterior ethmoid	-	-
Posterior ethmoid	-	-
Sphenoid	-	-
Maxillary	-	-
Osteomeatal complex*	-	-

Each sinus cavity is scored according to the extent of disease present.
0= clear , 1= partial opacification , 2= total opacification
** scoring for the osteomeatal complex: 0= clear , 2= occluded*
Total score ranges from 0 to 24, with maximum of 12 for each side.

H. pylori status was positive if 2 tests from 3 tests were positive[10,11].

We used the chi-square test and fisher exact test for statistical calculation. A P-value <0.05 was considered as significant.

RESULTS

In the study group, there were 37 patients (23 men and 14 women) with a mean age of 41.27 years (age range 16-76), and in the control group there were 38 patients (29 men and 9 women) with a mean age of 24.4 years (age range 17-59). Patient characteristics and distribution of co-existing disorders are presented in table II. Five out of 37 (13.5%) patients with nasal polyposis and one out of 38 (2.6%) control patients had classic symptoms of reflux (P-value=0.09). We estimated the incidence of seropositivity at 72.97% (27 patients) in the study group and 36.8% (12 patients) in the control group (P-value=0.000). PCR for H. pylori genetic material were negative in both control and study group patients. CLO test was positive in 24.3% (9 patients) of study group patients whereas it was negative in all of control patients (P-value=0.001). Only 8.1% (3 patients) of study group patients were positive for at least 2 tests (P-value= 0.115). The results of the different tests used for detection of H. pylori are shown in table III.

Tale-II. Patient characteristics

	Nasal polyp N=37 N(%)	Turbinate N=38 N(%)
Previous sinus surgery	2 (5.4)	-
Previous steroid therapy	16 (43.2)	1 (2.6)
Smoker	3 (8.1)	5 (13.2)
Asthma	12 (32.4)	1 (2.6)
Allergy	15 (40.5)	2 (5.3)
ASA intolerance	2 (5.4)	-
Classic reflux symptoms	5 (13.5)	1 (2.6)
Mean age (range)	41.27 (16-76)	24.4 (17.59)
Mean lund-mackay score (range)	17.8 (7-24)	-

The table shows characteristics of patients and parameters related.

Table-III. Different test results in 2 groups

Test	NP (N=37)	Control (N=38)	P-value
ELISA +	27 (72.97%)	12 (31.57%)	0.000
CLO +	9 (24.3%)	-	0.001
PCR +	-	-	-
2 test + (ELISA + and CLO)	3 (8.1%)	-	0.115

The above table depicts positivity of various tests in two groups

DISCUSSION

There have been reports about the association of gastroesophageal reflux (GER), chronic rhinosinusits (CRS) and nasal polyposis (NP)[10,11,12]. Because a significant number of GER patients are infected with H. pylori and the infection seems to disturb lower esophageal sphincter function, the hypothesis of a refluxate conveyed bacterial seeding of the supraesophageal mucosa is perhaps no too far-

fetched[10,15].

It has been suggested that the reflux of infected gastric juice into the nasophaynx may produce airway mucosal edema and inflammation the combined activity of the peptic-acid injury and local H. pylori infection[9,10].

Colonization by H. pylori has been found in dental plaque, saliva, tonsils and adenoid tissues. Direct transmission of H. pylori infection from the oral cavity to these structures might occur[6,7,9,10].

Karlidag et al. found that H. pylori DNA in 16.3% patients with otitis media with effusion by PCR assay[16].

There are some studies relating H. pylori and NP and CRS, but their results are both supporting, as well as opposing each other[6,7,10,15]. Some reports have suggested a 20-30% prevalence of H. pylori in the nasal cavity of CRS and NP patients[6,7].

Two studies were able to detect H. pylori in mucosa of patients having sinusitis with help of IHC method, but couldn't detect it in normal individuals. Morinaka detected H. pylori in sinuses of patients having sinusitis who had known H. pylori infection of stomach[10]. Halkis also detected H. pylori in 6 out of 15 Nasal polyp patients by CLO test, however tests were negative for patients with Concha Bullosa[12]. Dinis found H. pylori positive in mosuca of both normal and sinusitis patients but there was no difference in pepsin levels in nasal mucosa of case and control group[15].

In present study seropositivity of H. pylori in polyp group was 72.97% and in control group it was 31.57% ($p<0.05$). In similar studies seropositivity between two groups was variant but not statistically significant[6,12].

The advantages of serologic tests are that they are non invasive, relatively inexpensive. They are not affected by prior treatment with antibiotics or proton-pump inhibitors. However, the diagnostic significance of the ELISA test is limited because it can not discriminate between current and old infection[6,12]. It is stated that about 70% of the normal population is seropositive for H. pylori and can remain as asymptomatic throughout life[8,17]. A high prevalence of H. pylori in the serum is suggestive of other underlying reasons for H. pylori infection rather than NP, as is seen in our results, and so the relevance of such a positive result to H. pylori colonization of nasal and sinus mucosa is unknown.

For H. pylori detection, tissue Biopsy is superior to seropositivity.

In our study, similar to Halkis[12], CLO test was positive in 24% of nasal polyp patients whereas it was negative in control group. In a study using RUT (GUT Plus) test none of the mucosa of either group was positive for H. pylori[18]. In a study, GUT test was found not enough sensitive and was unreliable[19].

Cellini et al. could not find H. pylori in the nasal, mucosa of dyspeptic patients, although most of patients had H. pylori in the gastric mucosa[20].

Ozdek et al. found H. pylori DNA in 4 (33%) of 12 patients with chronic rhino sinusitis by PCR assay[7].

In our study PCR could not reveal H. pylori in either case or control group. The difference between these two studies needs to be defined.

Different methods can be used to detect H. pylori infection. Invasive technique include culture, biopsy, CLO test and PCR assay. Non invasive techniques include serology and the C-Urea breath test. The CLO test provides fast results and is easy to perform. The sensitivity of the CLO test for H. pylori has been reported to be 90.2% and specificity 92-100%, which is high[11,17,21]; the CLO test detects 95% of H. pylori infections within 24 hours. After 24 hours, late false positive reactions may be caused by growth of contaminating organisms[11].

On the other hand, the percentage of positive CLO test depends on the concentration of H. pylori. A weakly positive CLO test may occur when low concentration of H. pylori are present[10,11] or when partially degraded H. pylori shows decreased urease activity of H. pylori could be partially or completely inhabited by incubation with

antibiotics, whereas lignocaine does not interfere with the CLO test[10]. Therefore, occurrence of false positivity and even false negative in Rut results may occur.

Difference between results of various studies including present one can be explained as follows:

- Unlike the gastric environment, there are other potential contaminating organisms in the nasal cavity; this contamination can result in false-positive results in CLO test and immunohisto-chemistry (IHC)[10,11].
- H. pylori has a patchy distribution[10,11]. A patchy distribution of H. pylori may explain the positive IHC results with a negative result on PCR or CLO test.
- Maybe the numbers of H. Pylori present were less than those required for CLO test or PCR detection threshold. Also may be due to consumption of antibiotics (even before one month) or presence of unfavorable environment (viz. presence of other flora and mucociliary wave) H. pylori is transformed from bacillus to cocci form and so couldn't be detected by PCR, whereas IHC can even detect H. pylori after undergoing degenerative changes[11].
- H. pylori is sensitive to various antibiotics, but use of antibiotic in the patients with Rhinosinusitis is hard to avoid[10,11,20]. H_2 blockers and proton pump inhibitor (PPI) inhibit the growth of H. pylori[10]. Therefore, these compounds can influence the detection of H. pylori in the stomach; However, whether these drugs influence H. pylori detection in the nasal and sinus cavities in unknown.
- Lidocaine inhibits the growth of H. pylori[12], but lidocaine for local Anesthesia was not avoided in the present study. Epinephrine was mixed with lidocaine to cause vasoconstriction and minimize bleeding during surgery. Mucosal vasoconstriction would increase contact with oxygen, but inhibits the growth of H. pylori and accelerates it shape change from the bacillary to the coccoid from[10,11].

Although culture is theoretically the gold standard for detection of bacterium, it is technically difficult and is reported to be the least sensitive method for detection of H. pylori[16].

The advantage of PCR is an excellent sensitivity and specificity (96,7 and 100% respectively), Various studies have mentioned PCR to be at least as sensitive as culture and an important tool as its replacement[16,17,21,22].

The results of all these studies suggest that serological tests, light microscopy, rapid urease test and IHC test may provide false-positive results for H. pylori detection in upper-aerodigestive tissues. Thus, the best appropriate way to show the H. pylori present in tissues is culturing and PCR[16,17,21,22]. Cellini and Burduk recommended the use of more sensitive detection techniques such as the PCR as culture replacement[20,23].

One of the theories for justification of presence of H. pylori in nasal cavity is reflux of bacterium from stomach [6,7,10,11,16], even though association between reflux and H. pylori is itself suspected as in many studies there was negative association between reflux and its severity with H. pylori[24,25]. Although in our study there is no significant association between reflux and nasal polyposis than control group, it is still higher (13.5 and 2.6% respectively). So we can elaborate that reflux in patients with nasal polyps is high, although H. pylori is less.

In our study average severity of polyposis by using lund-macky system was 17.8 whereas it was 13.6 and 13.1 in studies conducted by Hopkins and Kim respectively[12,13]. In study by Kim, in spite of high prevalence of H. pylori in chronic sinusitis, severity of sinusitis was not related to presence of H. pylori[12]. More severity in our patients may be due to changes in anatomy of mucosa and environment of the nasal mucosa which inactive H. pylori or leads to its degeneration which in turn cause PCR to give negative results, while CLO test in spite of degenerative changes of H. pylori at least shows weakly positive[10].

We believe that results of RUT and serology were false

positive and on basis of PCR we say that H. pylori is not present in polyposis nor in normal nasal mucosa. In spite of all this it is possible that H. pylori alone and primarily or in association with reflux can be one of the several causes leading to polyposis by activating immune system and subsequent inflammation.

CONCLUSIONS

We could not detect H. pylori in nasal polyps nor in nasal mucosa. In most of studies all of the patients having classic symptoms of reflux were considered as positive the same disease, and were not objectively diagnosed.

It seems that diagnosing H. pylori in nasal mucosa is different than in stomach. Even it is possible that presence of H. pylori in stomach may hinder its presence in nasal mucosa. So it is suggested studies having more sample volume, objective analysis of reflux, concomitant measurement of H. pylori in stomach & nasal cavity and control of sinusitis be conducted. A sample should first be screened by RUT, if positive, then undergo PCR. This method would provide good cost benefits also.

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The optimist sees the rose and not its thorns; the pessimist stares at the thorns, oblivious of the rose.

Khalil Gibran