Detection of *Helicobacter pylori* in Nasal Polyps

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Abstract To detect the presence of *Helicobacter pylori* in nasal polyps. A case–control study was conducted enrolling 35 patients with nasal polyps (cases) and patients undergoing septoplasty (controls). Fresh tissue samples were used for urea broth test and imprint cytology, while formalin fixed tissue sections were used for morphology, special stains and immunohistochemistry for *H. pylori*. Fresh stool samples from both groups were tested to correlate the gastrointestinal status. *H. pylori* was detected in 40.0 % (14/35) of cases and 8.5 % of controls (3/35) ($p = 0.004$) by immunohistochemistry. Amongst cases, eight were positive with urea broth test, six with imprint cytology (Giemsa stain), three with H & E, and nine with modified McMullen's stain. Hyperplasia of the lining epithelium and lymphoid aggregates were significantly noticed in nasal polyps positive for *H. pylori*. Stool antigen test was positive in subjects who were positive for *H. pylori* in the nasal mucosa. There appears to be an association between *H. pylori* and nasal polyps. Immunohistochemistry is more sensitive and specific method to detect *H. pylori*.

H. pylori induced inflammatory tissue reaction pattern indicates a possible causal association. Further studies are needed to prove the causal relationship between *H. pylori* and nasal polyps.

Keywords Nasal polyps · *Helicobacter pylori* · Chronic rhinosinusitis · Imprint cytology · Giemsa stain · Urea broth test · Modified McMullen's stain · Immunohistochemistry · Stool antigen test

Introduction

Nasal polyps are overgrowths of chronically inflamed nasal and paranasal sinus mucosa affecting about 1–4 % of the general population [1, 2]. It is a multifactorial disease rather than a single disease entity. Despite studying various etiological factors such as allergy, genetic factors, infections and inflammatory reactions, the exact mechanisms involved in their causation are largely unknown.

In recent years, gastroesophageal reflux (GER) has been studied in the pathogenesis of chronic rhinosinusitis (CRS) with formation of nasal polyps [3, 4]. Since a significant number of GER patients are infected with *H. pylori*, nasal mucosa may be indirectly exposed to the bacterium [5]. However, the role of *H. pylori* in the pathogenesis of nasal polyps is yet to be elucidated. If a causal association between nasal polyps and *H. pylori* is confirmed, it may produce a major impact in the management of the disease and may prevent recurrences.

H. pylori infection is more common in developing countries. Apart from being a major etiological agent in chronic gastritis, peptic ulcer disease, and various gastric malignancies [6, 7], an association between *H. pylori* and certain extra-gastric manifestations such as haematological

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disorders, cardiovascular and respiratory disorders, neurological disorders, diabetes mellitus, ear and eyes diseases, and head and neck malignancies has also been noted [8, 9]. It has also been identified in human dental plaques, saliva, in oral lesions and ulcers, and in adenotonsillar tissue [10, 11]. Later studies have detected *H. pylori* in nasal mucosa of chronic rhinosinusitis [12–15] and nasal polyps [16–24] with variable results, which can be attributed to different sample size and detecting methods.

In the present study, we attempted to detect *H. pylori* in nasal polyps by different methods such as urea broth test, imprint cytology, histology, and immunohistochemistry (IHC). Simultaneously, the tissue reaction pattern associated with the presence of *H. pylori* in nasal polyps was also studied. Stool antigen test of the same subjects was done to correlate active gastrointestinal colonization with *H. pylori*.

Materials and Methods

Cases and Controls

This was a case control study conducted from November 2011 to December 2012 in the Department of Pathology, Microbiology, and Otorhinolaryngology of our institute after approval from Institutional Ethics Committee (Human Research). Informed consent was taken from patients before collection of samples.

35 cases with nasal polyps planned to undergo endoscopic sinus surgery and 35 controls planned for septoplasty were selected from Otorhinolaryngology in-patient department. The subjects were consecutively included in the study without any preference to a particular age group or sex and were subjected to thorough history, clinical examination, and relevant laboratory investigations. Every patient was asked a history of GER (acid regurgitation, heartburn) in particular.

Histologically confirmed cases of nasal polyps were included as cases and controls showed unremarkable nasal mucosa. Patient on antibiotics <1 month before surgery were excluded from the study. Tissue from nasal polyps in cases and lateral nasal wall mucosa from controls were collected separately under sterile conditions. Stool samples from same subjects were collected 1 day before surgery.

While interpreting various bacteriological tests, the history of GER, and results of stool antigen test were unknown to the pathologist.

Sample Processing

A part of the biopsy sample was incubated at 37 °C for 2 h in a sterile tube containing 1.5–2 ml of freshly prepared

urea broth. Any change in color from yellow to pink was interpreted as a positive reaction.

For Imprint cytology, air-dried touch smears were made from fresh tissue and stained with 2 % modified Giemsa solution overnight at room temperature. Grey-blue ‘S’ shaped bacteria were interpreted as *H. pylori* when examined under 100× objective.

Biopsy samples were subsequently fixed in 10 % buffered formalin, processed for paraffin embedding, and stained.

H & E Stain

Sections were used for tissue diagnosis, visualization of the bacteria and any specific morphological changes associated with *H. pylori* infection.

Modified McMullen’s Stain (Previously Called as Gimenez Stain)

The sections were stained with working carbol fuchsin for 2 min and then washed well in tap water. Subsequently, malachite green was added for 15–20 s and washed thoroughly with distilled water. *H. pylori* stained as red-magenta curved bacilli against a green background. A known positive control of *H. pylori* infected gastric mucosa was used for validation of the technique.

Immunohistochemistry

3–4 µm thick sections from the tissue blocks were deparaffinized and brought to water. Citrate based antigen retrieval and inactivation of endogenous peroxidase was done. The sections were subsequently incubated with anti-*H. pylori* rabbit polyclonal antibody (Cell Marque, California, USA) as per the manufacturer’s instructions. Known positive control of *H. pylori* infected gastric mucosa and negative control samples were included in each staining batch. Sections were subsequently studied under 100× objective.

Helicobacter pylori antigen was detected in stool using the principle of lateral flow immunoassay through ImmunoCard STAT! HpSA (Meridian Bioscience, Cincinnati, USA) as per the manufacturer’s instructions. Appearance of a pink-red line in the T- window indicated positive result after the appearance of a blue band at the control line.

Statistical Analysis

Apart from descriptive analysis, Fisher’s exact test and Pearson’s Chi square test (as appropriate) was applied for the study. *p* value <0.05 was considered statistically significant. Various morphological parameters were analyzed

using Pearson's Chi square test/Fishers exact test (as appropriate) in cross tabulation form with the two groups to find any significant association between *H. pylori* infection and the tissue reaction it produces. Statistical analysis was performed by SPSS version 20.0.

Results

Mean ages in case and control groups were 32 ± 11.0 and 28.2 ± 8.0 years, respectively. The male:female ratio was 1.3:1 and 10.6:1 for cases and controls, respectively. The subjects of our study were mostly from lower socioeconomic group. 7/35 (20 %) patients with nasal polyps and 6/35 (17.1 %) control patients had associated classic symptoms of reflux. Out of these 7 patients with nasal polyps and reflux symptoms, 4 were positive for *H. pylori*. Of the 6 control patients with symptoms of reflux, only 1 was positive for *H. pylori*. Patient characteristics and relevant parameters are presented in Table 1.

14/35 (40.0 %) cases and 3/35 (8.5 %) controls were positive for *H. pylori* ($p = 0.004$) with immunohistochemistry.

Table 1 Patient characteristics and relevant parameters

Parameters	Cases ($n = 35$)	Controls ($n = 35$)
Mean age $\pm$ S.D.	32.0 ± 11.0	28.2 ± 8.0
M:F ratio	1.3:1	10.6:1
Recurrence	2	0
Asthma	1	0
Aspirin intolerance	0	0
Classic reflux symptoms	7	6

The results of the various tests for cases and controls are depicted in Fig. 1. The modified Giemsa stained smears showed curved, S-shaped, grey-blue bacterium with ciliated columnar cells, mucin, abundant eosinophils, mast cells and few lymphocytes in the background (Fig. 2a). H & E stained sections showed *H. pylori* on the surface of the epithelium and in the mucus in 3 (8.5 %) cases. Modified McMullen's stain colored the bacteria magenta with a green background staining and was positive in 9 (25.7 %) cases (Fig. 2b). Amongst controls, only 3 (8.5 %) were positive for *H. pylori* with immunohistochemistry. Immunohistochemistry was a more sensitive and specific method for identifying *H. pylori* (Fig. 2c) in our study and a comparison between all detecting methods is shown in Table 2.

Various morphological findings were studied for the tissue response as a result of *H. pylori* in both cases and controls (Table 3). Hyperplastic pseudostratified ciliated columnar epithelium and dense lymphocytic infiltration forming lymphoid aggregates was seen more in *H. pylori* positive polyps (Fig. 2d) compared to *H. pylori* negative polyps and the results were statistically significant ($p = 0.03$, $p = 0.00$, respectively). Although, fibrosis was also seen more commonly in cases positive for *H. pylori* as compared to negative ones, the association was not significant ($p = 0.07$). Findings like submucosal edema, increased mucin/goblet cells, and dense eosinophilic infiltration were not statistically significant between *H. pylori* positive and negative cases. For controls, *H. pylori* positive patients did not show any remarkable changes in their mucosa. The sections showed a normal nasal mucosa with pseudostratified ciliated columnar epithelium, few submucosal glands, minimal inflammation, and underlying hyaline cartilage similar to *H. pylori* negative controls.

Fig. 1 Flowchart depicting the various tests and results of the study

Fig. 2 Photomicrograph showing results of various tests performed on the cases. **a** Imprint smear (nasal polyp) with presence of curved, S-shaped blue-grey bacteria *Helicobacter pylori* in the background (arrows) (modified Giemsa $\times 1000$). **b** Section from nasal polyp with few *Helicobacter pylori* adhered to the epithelium (circle) of the nasal mucosa. The bacteria stained pink-magenta on a green-blue background, identified as a curved, S-shaped organism (modified McMullen's stain $\times 1000$). **c** Nasal polyp stained with anti- *H.*

pylori antibody with numerous *H. pylori* adhered to the epithelium. The bacteria stained brown on a blue background with a curved, S-shaped morphology (circle). Inset shows *H. pylori* positive gastric mucosa (arrow) taken as a positive control (DAB chromogen $\times 1000$). **d** Section from a *H. pylori* positive case (nasal polyp) with hyperplastic pseudostratified ciliated epithelium and lymphoid hyperplasia (stars). However, identifying *H. pylori* in nasal polyps is difficult in this stain (H&E $\times 40$)

Table 2 Comparison between different tests for illustrating positive nasal polyps ($n = 14$)

	IHC	Urea broth test	Imprint cytology	H & E	Modified McMullen's stain
Positive	14	8	6	3	9
Negative	0	6	8	11	5

Table 3 Comparison of presence of various morphological parameters in *H. pylori* positive and *H. pylori* negative cases (nasal polyps)

Morphological parameters	<i>H. pylori</i> positive ($n = 14$)		<i>H. pylori</i> negative ($n = 21$)		<i>p</i> value
	Present	Absent	Present	Absent	
Hyperplastic epithelium	6	8	2	19	0.03
Edema	4	10	12	9	0.16
Fibrosis	7	7	4	17	0.07
Increased mucin/goblet cells	7	7	9	12	0.67
Dense lymphocytes	13	1	4	17	0.00
Dense eosinophils	2	12	9	12	0.13

Stool antigen test was positive in 19 out of 35 cases (54.2 %) out of which 14 were positive for *H. pylori* in their nasal mucosa. Similarly, amongst controls 12 out of

35 controls (34.2 %) were positive for stool antigen test out of which 3 were positive for *H. pylori* in their nasal mucosa.

Discussion

Helicobacter pylori has been classified as a class I carcinogen in humans by the Working Group of the World Health Organization International Agency for Research on Cancer [25]. Apart from its role as a factor for predisposition to several gastric and extragastric manifestations, a possibility of *H. pylori* in pathogenesis of nasal polyps has been suggested by various studies in the literature.

There are three hypotheses by which *H. pylori* reach the nasal cavity [26]. First, nasal cavity may be a reservoir of *H. pylori*. Second, oral cavity is a reservoir and the bacteria reach sinonasal cavity by oronasal reflux. Third, stomach is a reservoir and bacteria reach sinonasal cavity by gastroesophageal reflux. We found an increased incidence of GER in *H. pylori* positive cases. A possible pathogenesis of nasal polyps has been explained by poor mucociliary clearance of *H. pylori* in and around nasal polyps and the inflammation induced by the bacteria leading to epithelial cell proliferation [14]. We also found increased evidence of inflammation and epithelial proliferation in *H. pylori* positive cases.

The mean age for cases in our study was approximately 32 years. This was in accordance with the studies by Lathi et al. [27], and Zafar et al. [28] where second to fourth decade was most commonly affected. However, Chaaban et al. [29] showed an increased incidence in older age group with a mean onset of 42.5 years. Nasal polyps had slight predilection for males in our study, (male:female ratio of 1.3:1). It was similar to various studies [27, 28] but a study from Nigeria showed female preponderance (M:F = 1:1.2) [30]. There was a marked gender difference in controls (M:F = 10.6:1). Previous studies have also shown preponderance of males for the procedure of septoplasty [31].

Various invasive and non-invasive techniques are used to detect *H. pylori* infection. It is difficult to diagnose *H. pylori* when it is present in low numbers, in unusual forms, and in patchy or intermittent distribution. So, multiple methods of detection may be necessary to reach a diagnosis. Table 4 shows various studies published on the association between *H. pylori* and nasal polyps. The results of the studies varied according to their different sample size and detection methods. Some but not all studies have

Table 4 Various studies on association between *H. pylori* and nasal polyps

References	Year of publication	Samples	Sample size	Tests	Results positive (%)
Morinaka et al. [12]	2003	CRS	11	PCR, Urease test, Culture, IHC	18.1
Ozdek et al. [13]	2003	CRS	12	PCR	33.3
Kim et al. [14]	2007	CRS	48	RUT, IHC	25.0
Nikakhlagh et al. [15]	2015	CRS	50	PCR	18
Koc et al. [16]	2004	Nasal polyps	30	ELISA	86.7
				IHC	20
Szczygielski et al. [36]	2005	Nasal polyps	61	Urease test	0
Cvorovic et al. [17]	2008	Nasal polyps	23	Modified Giemsa, Urease test	26.0
Al-Abbasi et al. [34]	2008	Nasal polyps	25	PCR	8
Ozcan et al. [18]	2009	Nasal polyps	25	IHC	0
				ELISA	24
				CLO test	4
Kaviani et al. [19]	2009	Nasal polyps	37	ELISA, RUT	8.1
Ozyurt et al. [20]	2009	Nasal polyps	32	PCR	59.4
Burduk et al. [21]	2011	Nasal polyps	20	PCR (<i>ureA</i>)	100
				PCR (<i>cagA</i>)	0
Noorbakhsh et al. [32]	2011	Nasal polyps	62	PCR	32.3
				ELISA	14.5
Nemati et al. [33]	2012	Nasal polyps	25	PCR, RUT, Culture	0
Al-Abbasi et al. [22]	2012	Nasal polyps	28	Modified Giemsa	35
Vceva et al. [23]	2012	Nasal polyps	35	PCR	28.5
				ELISA	85.7
Khademi et al. [24]	2012	Nasal polyps	37	PCR, RUT, ELISA	8.1

shown the association between *H. pylori* and nasal polyps, but none has established causation yet.

Ozdek et al. [13] and Nikakhlagh et al. [15] found *H. pylori* DNA in patients with chronic rhinosinusitis using PCR assay. Studies by Vceva et al. [23] and Noorbakhsh et al. [32] used both PCR and serology and concluded a possible role of *H. pylori* in emergence of nasal polyps. Burduk et al. [21] believed that PCR assay is an appropriate method for detection of *H. pylori* in biopsy specimens, as culture is difficult to achieve. However, Nemati et al. [33], Abbasi et al. [34], and Ozyurt et al. [20] could not yield a significant result employing PCR.

Optimal methods such as PCR to access *H. pylori* even in low count, small sample size, and unusual forms has not been attempted in our study as the technique is costly, complex and requires strict sterile conditions. Though culture is the gold standard procedure for detection of any bacteria, it is not the case with *H. pylori* because of its fastidious growth requirements, unusual forms and losing its viability when exposed to the environment [35].

To improve the sensitivity and specificity for *H. pylori* detection, we employed immunohistochemistry, which had an advantage of identifying the bacteria even at low counts, intracellularly, and in degenerated coccoid forms. Possible reasons for degenerated forms are long term use of antibiotics, unfavourable nasal environment with numerous other bacteria, and poor nutrition [14]. However, false positive staining of cilia of the nasal epithelium and contaminating bacteria may lead to misinterpretation of the results; negative controls must be included in each batch of staining.

Koc et al. [16] demonstrated *H. pylori* in 20 % of cases based on IHC but the results were not the same with ELISA. Serology has a limitation of not discriminating old and new infection; also, it cannot distinguish the infection present at different sites. Kim et al. [14] evaluated patients suffering from CRS using IHC and CLO (Campylobacter-like organism) test and found a significant statistical difference between the cases and controls ($p = 0.025$). Morinaka et al. [12] in their study showed significant positivity with PCR and IHC as compared to urease test and culture. However, Ozcan et al. [18] could not detect *H. pylori* in nasal polyps by IHC.

Urea broth test could detect eight positive cases when compared to IHC. A weakly positive or false negative urea broth test may be due to low concentration or the degenerated forms of *H. pylori*. Moreover, the preparation of urea broth requires aseptic environment and the shelf life is 5–7 days. It has to be freshly prepared before applying the test, making it time consuming. Szczygielski et al. [36] and Nemati et al. [33] could not prove any association between *H. pylori* and nasal polyps on the basis of urease test. Khademi et al. [24] and Kaviani et al. [19] reported false

positivity in their study due to some other bacteria with urease activity. Overall, it can be concluded that urease test cannot be used as the only detection method; it has to be supplemented with more sensitive and specific methods like IHC or PCR.

Imprint cytology with modified Giemsa could identify only six cases as positive. Imprint cytology is a rapid and easy test and does not damage the biopsy material as also stated by Misra et al. [37], and Cubukcu et al. [38], in their experience with gastric biopsies. Al-Abbasi et al. [22] and Cvorovic et al. [17] got 35 and 26 % positivity in their study using modified Giemsa staining on nasal polyps. However, we found that low density of *H. pylori* in nasal polyps, bacterial contamination, and minimal contrast staining could yield erroneous results.

H & E identified only 21.4 % positivity and the possible reason behind low rates of *H. pylori* detection might be due to contaminating organisms, luminal debris, low density, and degenerated forms.

Special stains are always needed to detect low levels of infection and to see the characteristic morphology of *H. pylori*. Positivity with Modified McMullen's stain was 64.2 % when compared to IHC. Modified McMullen's stain is inexpensive, fast, and gives a good contrast and a better idea of morphology when it is performed well. However, it is difficult to standardize as the preparation of the stain and the timings of the staining are crucial which lead to both intra-batch and inter-batch variability, similar to the observations of Rotimi et al. [39].

With the comparison of the various test results, immunohistochemistry was found to be more sensitive and specific than any other tests performed in the study. Moreover, detection by immunohistochemistry is specific for *H. pylori* species as per the manufacturer's guidelines (Cell Marque, California, USA).

Besides the special and immunohistochemical tests, we also studied the histopathological changes in nasal polyps associated with the presence of *H. pylori*. The studies in the past have not discussed this important issue, which can point towards a pathogenetic role of *H. pylori* in causation of nasal polyps and not a mere colonization. In our study, we found a significant association of lymphoid aggregates and hyperplastic epithelium with *H. pylori* positive cases. A significant association of lymphoid aggregates with *H. pylori* positive gastric mucosa in chronic gastritis patients is already established [40]. It has been suggested in relation to gastric mucosa that *H. pylori* produces interleukin-1 β , which contributes to reactive hyperplasia of the epithelium, so a similar mechanism in nasal polyps may also be responsible [41].

To correlate with the gastric status of the patients, we did stool antigen test, which is a non-invasive, rapid, and technically easy procedure to perform. The subjects that

were positive for *H. pylori* in the nasal mucosa showed positivity in their stools also, thus suggesting a possible correlation.

There were few limitations in our study. First, for these types of epidemiological studies a larger sample size should be included in the study. Second, though PCR is the gold standard test for detection of *H. pylori*, we did not have access to the procedure thus IHC was used as gold standard. Third, to correlate with the gastrointestinal colonization by *H. pylori*, gastric biopsy must be performed. We substituted this part by relying on stool antigen detection.

Conclusions

There was a significant correlation between nasal polyps and presence of *H. pylori* in sinonasal mucosa. When present, morphological findings like hyperplasia of the epithelium and lymphoid aggregates are the significant changes in the *H. pylori* positive nasal polyps. Also, there exists an association between presence of *H. pylori* in nasal mucosa and gastrointestinal tract. However, to comment on whether there exists an association or a causal relationship, more epidemiological studies based on larger sample size are necessary.

Compliance with Ethical Standards

Conflict of interest None.

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