Original Research Paper Pathology

SIGNIFICANCE OF IMMUNOHISTOCHEMISTRY IN DETECTION OF H.PYLORI IN NASAL POLYPS.

DrSushma S Nigudgi Resident, Department of pathology,M.R Medical College, Kalaburagi

DrAnuradha G Patil HOD and Proffesor, Department of Pathology, M.R Medical College, Kalaburagi

ABSTRACT

BACKGROUND- *Helicobacter pylori is recognized as an important human pathogen associated with peptic ulcer disease, gastric cancer, and has high prevalence of infection worldwide. Recently this organism was detected in tonsillar and adenoid tissue, as well as in nasal mucosa of patients with chronic rhinosinusitis and nasal polyposis.*

AIM- *1)To study the presence of H.pylori in nasal polyps. 2) To study and compare the role of immunohistochemistry in the detection of H.pylori.*

METHODS - *30 nasal polypectomy specimens studied and compared to detect the presence of H.pylori by urease test (CLO), Giemsa stain and Immunohistochemistry using H.pylori antibody.*

RESULTS- *H-pylori was detected in nasal polyps by Urease test in 6.66% cases and by Giemsa stain in 16.66%, But by immunohistochemistry in 26.66% (8/30) cases significantly(P< 0.05).The 6/8 IHC positive cases were inflammatory polyps.*

CONCLUSION- *1) In present study significant association of H.Pylori is detected pointing towards its role in etiopathogenesis of nasal polyps.*
2)Treatment of H.Pylori may help in nasal polyposis especially in recurrence.
3) IHC is significantly better method for detection of H.Pylori than urease test(P<0.05) and can be considered cost effective for detection of H.Pylori in recurrent nasal polyposis.
However larger epidemiological studies would be needed

KEYWORDS : Nasal polyp, H.pylori, Immunohistochemistry

INTRODUCTION-

Nasal polyp is a common chronic inflammatory disease. It is benign pedunculated masses of nasal or sinonasal mucosa that is histologically characterized by infiltration of inflammatory cells and large quantities of extracellular edema. It causes considerable morbidity such as nasal obstruction, rhinorrhea and sleep disorders[1]. It affects about 1-4% of population with no difference in ethnic groups[2]. It is a multifactorialdisease[3]and no single factor can explain the pathogenesis of nasal polyps but inflammation still remains the central major factor in all cases.

H. pylori have been established both in gastric and extragastric manifestations. Besides stomach, some other natural reservoirs are also described. Colonization of the bacterium in dental plaques, saliva, and adeno-tonsillar tissue has been established.[4]H. pylori has been isolated from nasal and maxillary sinus specimens from patients with chronic sinusitis, nasal polyps, chronic otitis media with effusion, and upper respiratory disorders. Contribution of H. pylori in etiopathogenesis of nasal polyps is possible either through direct colonization, through oro-nasal route, or by gastroesophageal reflux (GER). GER leads to hypoxic and acidic environment favoring H. pylori to grow. Immunohistochemistry staining is now recognized as the gold standard in detecting H-pylori because it is highly sensitive and specific method.Immunohistochemistry analysis has an advantage of identifying the bacteria even in coccoid and dormant forms which cannot be achieved by routine H and E staining5.Thus, this study aims to detect the role of H. pylori in nasal polyps and To study and compare the role of immunohistochemistry in the detection of H.Pylori, so that it might influence/modify the present treatment lines for nasal polyps and may reduce the burden of recurrence.

MATERIALS AND METHODS-

The present study was conducted in the Department of Pathology, MR Medical College, Kalaburagi. All the surgical specimens from patients operated for Nasal polyp were received for routine histopathological evaluation, from Basaveshwar Teaching and General Hospital attached to M R Medical College, Kalaburagi. A total of 30 cases were studied of which ,22 were functional endoscopic sinus surgeries (FESS) and 08 were functional endoscopic sinus surgeries (FESS) with septoplasty done.

All the specimens were selected irrespective of age, sex and socioeconomic status. Urease test was done on fresh specimens. All specimens were fixed in 10% formalin solution and paraffin blocks were prepared which were cut at 4-5 microns thickness. They were subsequently stained with hematoxylin and eosin, Giemsa stain, and Immunohistochemical marker(AR442-5RE Helicobacter pylori polyclonal rabbit antibody).

Sample collection procedure:

- Specimens of nasal polyps were collected separately in empty container under sterile conditions and were brought immediately to the laboratory at room temperature.

a) Urease test: Each specimen was subjected to urease test. The medium used for the test was urea broth. The technique was:

- Urea broth consists of urea, phosphates, phenol red (pH indicator), and yeast extract in 1000 ml of distilled water. All the constituents were mixed well and final pH was adjusted to 6.8 ± 0.2 at 25°C. The prepared medium was yellowish-orange coloured clear solution. Yeast extract provides nitrogen and vitamin for bacteria, and phosphates serve to buffer the medium.
- 1.5 to 2 ml medium was dispensed in sterile test tubes and tissue sample was inoculated in the tube. Incubation was done at 37°C for 24 hours.
- The test was read at 1.5, 4, 18 and 24 hours, and was considered positive if the colour of the medium changed from yellow to pink or magenta.

b) Histological examination: Tissue samples were fixed in 10% buffered formalin, routinely processed, and 3-4 µm thick paraffin embedded sections were taken from each tissue block for Haematoxylin and Eosin staining, special staining (Giemsa stain) and immunohistochemistry (IHC) on polylysine coated slides after deparaffinizing and rehydrating through graded alcohols to distilled water.

c) Immunohistochemistry procedure : To improve the specificity of the results, immunohistochemistry was employed in the study as the gold standard. For immunohistochemical demonstration of Helicobacter pylori, 3-4 µm thick sections from the tissue blocks were deparaffinized and brought to water. Thereafter, the sections were rinsed in distilled water and heated in microwave oven for 20 minutes in 0.01 M citrate buffer (pH-6.0). After microwave oven heating, the slides were rinsed in PBS buffer (pH-7.2) for 15 minutes after being brought to room temperature. Endogenous peroxide was blocked by 4% H2O2 for 30 minutes followed by 3 washings of 5 minutes each with PBS buffer. The sections were incubated with the polyclonal anti-H. pylori antibody (rabbit polyclonal antibody 215A-75 from Cell Marque). The antibody was used in 1:50 dilution. The slides were then incubated overnight at 4oC in a refrigerator. After bringing the slides to room temperature the next morning, the sections were washed thrice for 5 min with PBS. They were treated with the Polyscan HRP label for 30 minutes and then were given 3 washes with PBS buffer, each for 5 minutes. Colour was developed using diaminobenzidinetetrahydrochloride as a substrate, counterstaining was done with Harris Haematoxylin followed by washing in distill water. The sections were blotted, air dried, and mounted with DPX. The sections were examined in oil immersion and the results were interpreted as positive or negative

The statistical analysis for correlation among these parameters was determined usingthe Pearson chi-square test. Significance was assumed at p-value less than 0.05.

RESULTS –

The present study includes 30 cases of nasal polyps, 14 were males and 16 were females, their age ranged between 7 to 55 years (mean 26.46 years). Most of the patients presented with the history of nasal obstruction, followed by nasal discharge, anosmia, sinusitis and headache. 5 cases (16.66%) gave positive history of gastroesophageal reflux symptoms and 3 cases (10%) had recurrence out of total 30 patients. Histologically 19 cases were Inflammatory polyps and rest 11 cases were Eosinophillic edematous polyps. Urease test was done in all cases of nasal polyps showed positive for H.pylori in 02 cases (6.66%), giemsa stain showed positive for H.pyloriin 05 cases (16.66%), and Immunohistochemistry with H.pylori antibody showed positive in 08 cases (26.66%) which was statistically significant(P-value <0.005%) than compared to other tests. Table 1 showing results of different tests conducted on nasal polyps for the presence of H.pylori.

Table 1- Showing results of different tests conducted on nasal polyps for the presence of H.pylori

Study/parameters	Positive	Negative	Total	
Urease test	02(6.66%)	28(93.33%)	30(100%)	
Giemsa stain	05(16.66%)	25(83.33%)	30(100%)	
IHC with H.pylori antibody	08(26.66%)	22(73.33%)	30(100%)	P-value <0.05

Figure 1- Urease test for H.pylori (Positive- magenta pink colour, negative- yellow colour)

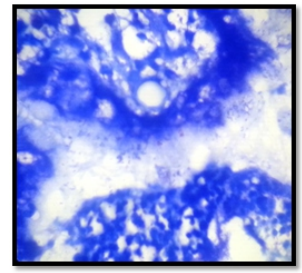

Figure 2- Giemsa stain- showing spiral bacterium on lining epithelium of nasal polyp- positive for H.pylori

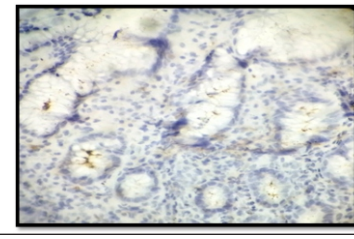

Figure 3- Gastric biopsy showing positive for H.Pylori by IHC (H.Pylori polyclonal antibody) used as a control in this study

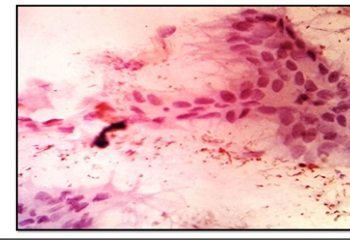

Figure 4- Nasal polyp positive for H.Pylori by IHC (H.Pylori polyclonal antibody)

Discussion-

Nasal polyps were first described more than 3000 years ago and comprise most common group of mass lesions encountered in the nose. Despite this long history and frequent occurrence, many questions still exist with regard to incidence, pathogenesis and treatment[6]. It affects about 1-4% of population with no difference in ethnic groups[3] Chronic rhinosinusitis plays a foremost role in the development of nasal polyps. Other factors which play an important role in etiopathogenesis are allergic conditions like asthma, rhinitis including hay fever, aspirin intolerance, allergic fungal sinusitis, nasal mastocytosis, certain syndromes and genetic conditions like Churg-Strauss syndrome, Young's syndrome, cystic fibrosis, and Kartagener's syndrome.[7]

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.[8] Apart from its role as a factor for predisposition to several gastric and extragastric manifestation, a possibility of *H. pylori* in pathogenesis of nasal polyps has been suggested in the literature.[9] H. pylori is the most prevalent chronic infectious etiology in humans worldwide. It is more common in developing than in developed countries. There have been reports in the literature on the association of H. pylori and nasal polyps, but their results have been contradictory. A possible role of H. pylori in the pathogenesis of nasal polyps has been explained that inflammation induced by the bacteria leads to epithelial cell proliferation. Three hypotheses by which H. pylori can reach the nasal cavity are recommended. First, nasal cavity is a reservoir of H. pylori. Second, oral cavity is a reservoir and the bacteria reaches sinonasal cavity by oronasal reflux. And third, stomach is a reservoir and bacteria reaches sinonasal cavity by gastroesophageal reflux[10].

Various studies have been done to detect H.pylori in nasal polyp

specimens using various invasive and non invasive techniques. Morinaka S et al(2003) found H.pylori in sinus mucosa of some patients with chronic sinusitis by urease test and got 63.6% positive results by IHC studies out of 11 patients.[11]Koc C et al.(2004) reported that H.pylori was detected in six nasal polyp (20%) specimens out of 30 patients by Immunohistochemistry.[12] They reported that, H. pylori was found with increased prevalence in nasal polyps.Kaviani et al.(2009) reported 27 (66.2 %) versus 2(10 %) positive result by Elisa test for H. pylori antibody checking and rapid urease test in case & control group respectively, and they suggested significant relationship between H.pylori infection and nasal polyposis.[13]Jelavic et al (2012) reported 70% positive results by IHC studies out of 40 patients.[14] Another study done by Ahmed muhamad et al(2012) detected h.pylori in 10(35%) of 28 patients with nasal polyp.[15]However our study showed 26.66% (8/30) positivity for H.pylori in nasal polyps by immunohistochemistry using H.pylori antibody then compared to other tests which was statistically significant (P value <0.05).

To conclude 1) In present study significant association of H.Pylori is detected pointing towards its role in etiopathogenesis of nasal polyps.
2)Treatment of H.Pylori may help in nasal polyposis especially in recurrence.
3) IHC is significantly better method for detection of H.Pylori than urease test(P<0.05) and can be considered cost effective for detection of H.Pylori in recurrent nasal polyposis.
However larger epidemiological studies would be needed.

REFFERENCES

1. Ozcan C, Zeren H, Talas DU, Küçükoğlu M, Görür K. Antrochoanal polyp: a transmission electron and light microscopic study. Eur Arch Otorhinolaryngol2005;262:55-60. [15004706] [doi:10.1007/s00405-003-0729-1]
2. Larsen K, Tos M. The estimated incidence of symptomatic nasal polyps. ActaOtolaryngol. 2002;122(2):179–182
3. Hsu J, Peters AT. Pathophysiology of chronic rhinosinusitis with nasal polyp. Am J Rhinol Allergy. 2011;25(5):285–290
4. Atherton JC. The pathogenesis of Helicobacter pylori-induced gastro-duodenal diseases. Annu Rev Pathol. 2006;1:63-96.
5. Ashton-Key M, Diss TC, Isaacson PG. Detection of Helicobacter pylori in gastric biopsy and resection specimens. J ClinPathol. 1996;49(2):107-111.
6. Dykewicz MS, Hamilos DL. Rhinitis and sinusitis. J Allergy ClinImmunol. 2010;125 Suppl:S103-115.
7. Slavin R G, Nsal polyps and sinusitis. In Middleton E, Reed CE, Adikinson EFE, Yunginger and William, Allergy principle and practice Vol 2, New York:Mosby: 1993, P-1455-9
8. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC MonogrEvalCarcinog Risks Hum. 1994;61:1-241.
9. Al-Abbasi AM, Jasim AH. Association of Helicobacter pylori and Nasal Polyposis. The Iraqi Postgraduate Medical Journal. 2012;11(1):92-96.
10. F Khajeh, MH Motazedain, Z Saforpoor, MH Meshkibaf, B Miladpoor Iran Red Crescent Med J 2011; 13(6): 136-137.
11. Morinaka S, Ichimiya M, Nakamura H. Detection of Helicobacter pylori in nasal and maxillary sinus specimens from patients with chronic sinusitis. Laryngoscope 2003;113: 1557-63.
12. Koc C, Arikan OK, Atasoy P, Aksoy A. Prevalence of Helicobacter pylori in patients with nasal polyps: a preliminary report. Laryngoscope 2004;114:1941-4.
13. Kaviani M, Khademi B, Moosavi SA, Azar pira N, Ashraf MJ. Detection of Helicobacter pylori in nasal polyp using rapid urease test and ELISA. Iran Otolaryngology J 2009;20:189-96.
14. Jelavic B, Grgic'M, Cupric'H, Kordic'M, Vasilj M, Baudoin T. prognostic value of Helicobacter pylori sinonasal colonization for efficacy of endoscopic sinus surgery Eur Arch otorhinolaryngol. 2012;269:2197-2202.
15. Ahmed Muhamad Al-Abbasi, Aseel Hamid Jasim; association of Helicobacter pylori and nasal polyposis; The Iraqi postgraduate medical journal. Vol.11 no.1,2012.