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Helicobacter Pylori in Patients Suffering from Pulmonary Disease

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ABSTRACT

Background: Recently, research of indirect evidence suggested a possible association between *Helicobacter pylori* and pulmonary disease. This study aimed to determine if *H. pylori* could be detected in endobronchial specimens collected from patients undergoing bronchoscopy.

Materials and Methods: This prospective study was conducted on 34 consecutive patients with any type of lung disease undergoing bronchoscopy in which biopsy was required for their diagnosis. A written informed consent was obtained from all participants. Three bronchial mucosa biopsy samples were obtained using fenestrated biopsy forceps. One sample was used to determine urease activity, the second one for histopathological examination, and the third one for diagnosis. All subjects were fully informed regarding the gastroesophageal reflux disorder (GERD) Questionnaire.

Results: There were 34 patients with pulmonary diseases (12 males and 22 females, mean age 58.2 ± 18.2 years) out of which, 11 (32.4%) had GERD. No significant difference was found between the histopathological assay and GERD.

Conclusion: Our study found no direct evidence supporting the theory that *H. pylori* may cause pulmonary disease and no relation with GERD was detected. However, a possible indirect role could not be excluded. Further studies in patients with GERD and lung disease may reveal a potential pathogenic link between *H. pylori* and pulmonary disease. (**Tanaffos2011; 10(1): 31-36**)

Key words: *Helicobacter pylori*, Bronchoscopy, Lung disease, Gastroesophageal reflux disease (GERD)

INTRODUCTION

Helicobacter pylori infection of the gastric mucosa affects approximately 50% of the world's population (1). A review of previous literature

suggests that the prevalence of *H. pylori* infection is about 44.9% in general population and 47% in Iranian population(2). It seems to be the main cause of chronic antral gastritis and is strongly associated with peptic ulcer disease, gastric cancer, and gastric MALT-lymphoma (3-6). In the past few years, a variety of extra digestive disorders, including cardiovascular, skin, rheumatic and liver diseases,

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have also been associated with *H. pylori* infection (7,8). As regards respiratory diseases, an increased seroprevalence of *H. pylori* has been found in active bronchiectasis, chronic bronchitis and active pulmonary tuberculosis (9-12). Moreover, both chronic obstructive pulmonary disease and pulmonary tuberculosis are more prevalent in peptic ulcer patients than in the general population (13- 15). The activation of inflammatory mediators by *H. pylori* seems to be the common pathogenic mechanism underlying the observed associations (14). However, these studies did not directly demonstrate a correlation between lung infection and *H. pylori*.

It is well known that the prevalence of lung cancer in peptic ulcer patients increases 2 to 3 folds compared with findings in ulcer-free controls (16-21). The major factor underlying this association seems to be the impact of cigarette smoking on both diseases. However, a recent pilot study on a small number of patients showed that *H. pylori* infection, per se, might be implicated in lung cancer (21). It suggested that the prolonged release of gastrin and cyclooxygenase COX-2 in *H. pylori* infected patients might account for the stimulation of lung cancer growth and tumor neoangiogenesis (22). However, insufficient information is available on the prevalence of *H. pylori* infection in lung cancer patients.

The prevalence of gastroesophageal reflux disease in Iranian people was 39.7% (23). GERD could be another predisposing factor for the transport of *H. pylori* to the oropharynx and bronchial lumen.

Activation of inflammatory mediators and/or induction of autoimmunity may directly or indirectly play a role in respiratory diseases. For that reason, many recent studies paid attention to the association between *H. pylori* infection and various respiratory disorders but there is controversy regarding *H. pylori* infection affecting respiratory disorders directly or

indirectly.

Prevalence of *H. pylori* in Iran is higher than other searched locations. Other research studies were limited to one or two pulmonary diseases. Therefore, it seemed like we need further investigation to find a correlation between *H. pylori* infection and respiratory disorders. In this study, we assessed the presence of *H. pylori* in lung specimens of patients undergoing bronchoscopy in Hazrate-Rasoul Akram Hospital.

MATERIALS AND METHODS

Patients

We studied 34 consecutive patients with various respiratory disorders undergoing bronchoscopy. Biopsy was required for their diagnosis. Patients were excluded if they had:

- 1) Any co-existent cardiovascular disease
- 2) pregnancy
- 3) Acute severe asthma
- 4) High risk of bleeding, or were
- 5) Non- compliant

After choosing the understudy population, a written informed consent was obtained from all patients. The information included demographic characteristics; and clinical features of participants were assessed retrospectively.

Only cases undergoing bronchoscopy and biopsy were selected. Three bronchial mucosa biopsy specimens were obtained by fenestrated biopsy forceps (Olympus, Tokyo, Japan) for pathologic assessment from carina, using a fiberoptic bronchoscope (Olympus, Tokyo, Japan). One of these samples was used to determine urease activity as described below. We used Haematoxylin-eosin staining for histopathological evaluation of the presence of *H. pylori* in the second mucosa sample and the third specimen was used for histopathological diagnosis.

Biopsy specimens were crushed in neutral-

buffered formalin in a glass rod.

Patients' diagnosis was based on CXR (chest x-ray), HRCT (high resolution computerized tomography), pulmonary function test (if it is required) and pulmonary biopsies for pathologic evaluation.

Preparation of bronchial samples

We took one bronchial biopsy sample from the carina of subsegmental bronchi, because this area is mostly affected by pulmonary disease. Then, we evaluated the histological features by examining the haematoxylin-eosin stained sections. Sections were cut from each block with sterile and non-touch technique. Each section was 25 µm thick. We tried to use new blades to decrease contamination. Samples were incubated in xylene at 56°C for 10 minutes. They were washed once in 80% ethanol and once in acetone and left to dry for 1h. These sections were put in sterile 0.9% NaCl.

The histological sections were evaluated for the presence or absence of columnar epithelium, presence or absence of epithelial lesions, presence of inflammatory infiltrate in the lamina propria and presence of microorganisms.

Urease test

Immediately after collection, 1 biopsy was tested for urease activity by using the "hpfast" test (GI Supply, Camp Hill, PA) according to manufacturer's instructions. In brief, the biopsy was placed in an agar gel containing urea and 2 pH dye indicators: bromthymol blue and methyl red. Change of the agar color from yellow to dark green or blue within 24 hours was interpreted as a positive test result (23,24).

Ethics

The ethical committee of the hospital approved the study protocol. Informed consent was obtained from all patients.

Upper airway anesthesia for bronchoscopic examination was achieved by spraying 2 or 3 puffs of lidocaine 10% onto the throat. The bronchoscope (Pentax, Tokyo, Japan) passed trans-nasally or trans-orally into the trachea, avoiding any suction through the inner channel, and bronchial biopsy sample was taken at the end of the procedure.

GERD Questionnaire

The questionnaire covered all the demographic characteristics, medical history and major GERD symptoms and was filled out for all individuals. This questionnaire was validated and its reliability was estimated in Ehsani et al. study in Iran (23).

RESULTS

We studied 37 patients with any pulmonary disease undergoing bronchoscopy and biopsy samples were obtained from them. Three patients excluded from the study because their information was incomplete. The main clinical characteristics of understudy patients are summarized in Table 1. The mean age of participants was 58.2 ± 18.2 yrs. Men comprised 64.7% of the patients (22 subjects) and only 12 (35.3%) patients were smokers. Six (17.6%) of these patients inhaled opium and were addicts. Most patients were high school drop outs (22 subjects, 64.7%) and only one patient had a diploma. Thirty (88.2%) patients were housewives or had no occupational pollution exposure. Four (11.8%) patients were exposed to pollutants (i.e. were cooks or farmers).

Most patients (11 cases, 32.4%) had coughs and dyspnea, 7(20.6%) had coughs only; other patients had other symptoms such as chest pain and hemoptysis and one patient had no symptom. There were 3 (8.8%) cases of sarcoidosis, 5 (14.7%) cases of lung cancer, 3(8.8%) lymphoma, 2 (5.9%) bronchitis, and 10(61.8%) other diseases such as lung abscess, anthracosis, fungal infection, pneumonitis,

tuberculosis, bronchiectasis, bronchiolitis and interstitial lung fibrosis. But 10 patients had no definite diagnosis.

In this study, 11 (32.4%) patients had gastroesophageal reflux disorder (GERD) and 23 (67.6%) were free of it. There were no significant correlations between GERD and gender, occupation or smoking but a significant relationship was observed between GERD and educational status ($P=0.01$)

Table1. Clinical characteristics of the participants

Variable	
Gender (%)	
Male	22(64.7%)
Female	12(35.3%)
Age(mean \pm SD)	58.2 \pm 18.2
Smoking (%)	
Yes	12(35.3%)
No	22(64.7%)
Opium addiction (%)	
Yes	6(17.6%)
No	28(82.4%)
Employment (%)	
Pollution exposure	4(11.8%)
Other or unemployed	30(88.2%)
Educational status (%)	
Under Diploma	22(64.7%)
High school graduate	12(35.3%)
Location (%)	
Tehran	15(44.1%)
Other cities	19(55.8%)
Diagnosis	
Sarcoidosis	3(8.8%)
Lymphoma	3(8.8%)
Bronchitis	2(5.9%)
Lung cancer	5(14.7%)
Other	10(61.8%)

Bronchial biopsy analysis

We obtained a total of 34 bronchial samples. Of these 34 biopsies, only 32 samples were further processed for analysis. The remaining samples were excluded since they showed slaughtering/detachment of the surface epithelium.

No correlation was detected between histopathological evaluation and urease test for *H. pylori* in lung specimens. Mild inflammatory infiltrate of the lamina propria, composed of mature lymphocytes, was observed in 32 patients. In 32 cases, mixed acute and chronic inflammatory infiltrate was observed along with pathologic characteristics related to their disease. Giemsa staining was performed in all cases and none of them showed presence of *H. pylori*. No significant relationship was found between GERD and *H. pylori* in pathological staining and urease test.

DISCUSSION

The results of the present study did not support the pathogenic role of *H. pylori* in patients with lung disease in a clinically stable condition. Sample size in this study was small. The ability of *H. pylori* to reach lower airways along with the histopathological similarities between bronchiectasis and gastric peptic ulcer have prompted some investigators to raise the hypothesis that *H. pylori* might be implicated in the pathogenesis of some cases of bronchiectasis. Various authors have shown an increased prevalence of serum antibodies against *H. pylori* in patients with both chronic bronchitis and bronchiectasis (8-10). The presence of *H. pylori* in dental plaques, tonsils and adenoids suggest that the oral cavity is a reservoir of this microorganism. Another possible origin for *H. pylori* bronchial colonization is the gastric reservoir; in this case, GERD could be a predisposing factor for the transport of *H. pylori* to the oropharynx and bronchial lumen. To confirm this hypothesis, Ilvan et al.(25) and Gulhan et al.(26)

worked on the presence of *H. pylori* by performing rapid urease test and histopathological examination of bronchial samples with negative results but in these studies participants did not have gastric problem or GERD and they researched on patients with bronchiectasis only.

In our study, we tried to circumvent this limitation and we evaluated a more heterogeneous population that included patients of both gender, with a mean age of 58.2 years with any lung disease and evaluated GERD and smoking habit in them. The sample size was small and cases were not serious smokers. Therefore, our results confirmed those obtained by Ilvan et al.(25) and Gulhan et al.(26) that *H. pylori* was not found in bronchial biopsies of patients.

Our results suggested that the etiology of pulmonary diseases such as lung cancer, lymphoma, bronchiectasis and bronchitis was not directly related to bronchial *H. pylori* infection. However, products of *H. pylori* such as toxins, urease, catalase, phospholipases, alcohol dehydrogenase, hemolysine, platelet-activating factor, and mucolytic factor might play an indirect role in the pathogenesis of lung cancer and bronchiectasis or other pulmonary diseases that may have a correlation with *H. pylori*. Tsang et al, in his study showed that aspiration of acid along with *H. pylori* toxins present in gastric contents can lead to further damage in bronchiectatic airways and this could be a possible underlying mechanism for inflammation. This mechanism might be present in patients with GERD and bronchiectasis or lung cancer.

Previous studies showed controversial results regarding the relationship between *H. pylori* and smoking habit but in our study no correlation was found in this respect.

In conclusion, our study found no direct evidence that *H. pylori* may cause lung disease and no relation with GERD. However, a possible indirect role could not be excluded. Further studies on patients with

GERD and pulmonary diseases such as bronchiectasis, bronchitis, interstitial lung disease and lung cancer may reveal a potential pathogenic link between *H. pylori* and/or its products with lung disease.

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