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Helicobacter pylori Seroprevalence in Patients with Mild Asthma

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JUN, Z.J., LEI, Y., SHIMIZU, Y., DOBASHI, K. and MORI, M. *Helicobacter pylori Seroprevalence in Patients with Mild Asthma*. Tohoku J. Exp. Med., 2005, **207** (4), 287-291 —— *Helicobacter pylori* (*H. pylori*) is causally related to chronic active gastritis, peptic ulcer disease, primary low-grade B-cell gastric lymphoma, and is also a risk factor for gastric cancer. In addition, a high seroprevalence of *H. pylori* has been found in many extragastrointestinal disorders, including active bronchiectasis and chronic obstructive pulmonary disease (COPD). It appears that *H. pylori* has a close relationship with respiratory diseases, but data in the literature on the relationship between *H. pylori* infection and asthma are poor. We therefore investigated the relationship between them. In this study we evaluated 46 patients with mild asthma, 48 age- and sex-matched patients with peptic ulcer and 48 healthy control subjects. All enrolled subjects underwent a serologic test for *H. pylori* IgG and cytotoxin-associated gene-A (CagA) by enzyme-linked immunosorbent assay (ELISA). There was no significant difference in both anti-*H. pylori* IgG seropositivity ($p = 0.6580$) and anti-*H. pylori*-CagA IgG seropositivity ($p = 0.7183$) between the asthmatic and control subjects. In contrast, both anti-*H. pylori* IgG seropositivity and anti-*H. pylori*-CagA IgG seropositivity were significantly higher in peptic ulcer patients than these in asthmatic patients ($p < 0.01$). Despite the sero-epidemiological association of *H. pylori* infection with many inflammatory conditions, our data show no significant association between mild asthma and *H. pylori* infection. ———— asthma; *H. pylori*; CagA

Helicobacter (H.) pylori is a microaerophilic gram-negative spiral-shaped bacterium which is causally related to chronic active gastritis, peptic ulcer disease (Marshall and Warren 1984), and primary low-grade B-cell gastric lymphoma (Isaacson and Spencer 1993), and is also a risk factor for gastric cancer (Parsonnet et al. 1991). *H. pylori* infection is one of the most common bacterial diseases world-wide. A high *H. pylori* seroprevalence has also been found in many extragastrointestinal disorders, including coronary heart disease (Mendall et al. 1994), rosacea (Rebora et al. 1994), growth failure in childhood (Patel et al. 1994), active bronchiectasis (Tang et al. 1998) and chronic obstructive pulmonary disease (COPD) (Roussos et al. 2005). Recently, *H. pylori* has been identified in the tracheobronchial aspirates in mechanically ventilated patients,

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suggesting that it might cause ventilator-associated pneumonia (Mitz and Farber 1993). It appears that *H. pylori* has a close relationship with respiratory diseases.

It is well known that *H. pylori* colonization of the gastric mucosa stimulates the release of various proinflammatory substances, such as cytokines, eicosanoids and proteins of the acute phase (Crabtree 1998). Moreover, a cross mimicry between bacterial and host antigens exist in *H. pylori* infected patients (Negrini et al. 1996). Therefore, a pathogenetic link between *H. pylori* infection and diseases characterized by activation of inflammatory mediators and/or induction of autoimmunity might exist. Asthma is a very common respiratory disease and is characterized by the presence of cytokine-mediated airway inflammation and nervous system dysfunction or hyper-function (Ichinose 2003), leading to smooth muscle contraction, edema and progressive airway damage in some cases. We therefore hypothesize that there may be a correlation between *H. pylori* infection and asthma.

Subjects and Methods

Following a predefined protocol, between March 1, 2004 and March 31, 2005, three groups of subjects, who consented to provision of venous blood, were recruited consecutively in our hospital. One group consists of 46 mild asthmatic patients who were nonsmokers. The diagnosis of asthma was based on the criteria of the American Thoracic Society (1987). Severity of asthma was classified according to the National Institute of Health-World Health Organization guidelines (National Institute of Health 1997). Briefly, patients with mild asthma had symptoms twice a week or less often, with an FEV_1 of 80% of predicted value or greater, reversibility of greater than 15% with salbutamol, or a PC_{20} of less than 8 mg/ml histamine. They were receiving no regular medication but used inhaled β_2-agonists as needed for symptom relief. Exclusion criteria were: (1) an exacerbation of asthma in the preceding month, (2) prior *H. pylori* eradication therapy, (3) consumption of acid-suppressive drugs or antibiotics in the preceding 6 months, (4) a known history of gastrointestinal tract pathology, (5) a history of vagotomy or operations on the upper gastrointestinal tract. The other group consists of 48 sex, age, and socioeconomic matched nonsmoker patients who suffered from peptic ulcer (diagnosed by gastroduodenal endoscopy, 21 patients with duodenal ulcer, 27 patients with gastric ulcer), were also enrolled into this study. Exclusion criteria were: (1) previous gastric surgery and gastric malignancies, (2) patients under treatment with bismuth containing drugs, proton pump inhibitors, H_2 receptor antagonists and antibiotics during the last six months before the diagnosis, (3) coexistent/pre-existing systemic illness, and (4) alcoholism, nonsteroidal anti-inflammatory drug intake. Fourty eight sex, age, and socioeconomic matched nonsmoker controls were selected randomly from subjects who attended courses designed for public health education during the period of the study. Exclusion criteria for controls were: (1) a known history of asthma, and (2) a known history of gastrointestinal tract pathology.

This study was approved by the Gunma University Ethics Committee, and written informed consent was obtained from each participant. All subjects enrolled underwent an ELISA IgG serologic test for *H. pylori* and CagA protein diagnosis (Bio-Rad, Hercules, CA, USA) in accordance with the manufacturer's guidelines. The specificity and sensitivity of the serology test, validated in our local population were 95% and 85%, respectively. Positive, borderline, and negative results were assigned when the concentration of IgG antibodies against *H. pylori* was greater than 20, between 12.5 and 20, and less than 12.5 U/ml, respectively. Moreover, when the concentration of IgG Cag antibody was greater than 7.5, between 5.5 and 7.5, and less than 5.5 U/ml, the result was considered as positive, border line and negative, respectively.

Values are expressed as means ± S.D. The significance of differences between groups was assessed by unpaired Student's *t*-test for continuous variables and the x^2 test for proportions. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program (SPSS, LosAngeles, IL, USA), and *p* values were two-tailed analyzed. *P* values of less than 0.05 were considered statistically significant.

Results

Altogether 46 asthmatic patients, 48 peptic ulcer patients and 48 control subjects were recruited into this study. The demographic and serological parameters are shown in Table 1. There was no significant difference in age or sex distribution among the three groups. The serological parameters demonstrated that both anti-

TABLE 1. *Demographic and H. pylori serologic parameters*

Parameters	Asthma ($n = 46$)	Peptic ulcer ($n = 48$)	Controls ($n = 48$)
Age	51.2 ± 12.4	53.6 ± 13.6	50.3 ± 11.8
Male sex (%)	60.8	62.5	58.3
Anti-*H. pylori* IgG (+) (%)	58.7	89.6	54.2
Anti-*H. pylori*-CagA IgG (+) (%)	21.7	72.9	18.8

H. pylori IgG seropositivity and anti-*H. pylori*-CagA IgG seropositivity were significantly higher in peptic ulcer patients than in asthmatic patients ($p < 0.01$). However, there was no significant difference in both anti-*H. pylori* IgG seropositivity and anti-*H. pylori*-CagA IgG seropositivity between the asthmatic patients and control subjects ($p > 0.05$).

DISCUSSION

H. pylori infection is a major public health problem as it is widespread and frequent (Simsek et al. 2000), while data in the literature on the relationship between *H. pylori* infection and asthma are poor. Tsang et al. (2000) estimated the prevalence of *H. pylori* infection in a cohort of 90 patients with bronchial asthma and found that *H. pylori* seroprevalence did not differ significantly between asthmatic and control subjects (47.3% vs 38.1%, $p > 0.05$). The authors concluded that bronchial asthma might not be associated with *H. pylori* infection. As far as we know, the study of Tsang et al. (2000) is the only report concerning the association between *H. pylori* infection and asthma.

It is well known that age, sex and socioeconomic status are related with both *H. pylori* infection and risk of developing peptic ulcer (Feldman et al. 1998). Thus in our study patients with asthma and peptic ulcer were well matched with control subjects for these parameters. Tobacco use could be another confounding factor, but the data on the relation between *H. pylori* infection and smoking habits are controversial. The prevalence of *H. pylori* infection in smokers has been variously reported as low (Ogihara et al. 2000), normal (Brenner et al. 1997), and high (Parasher and Eastwood 2000). As the relation between smoking and *H. pylori* infection has not been clarified yet, in all three groups we recruited the nonsmokers in order to eliminate the disturbance of tobacco.

The study conducted by Tsang et al. (2000) showed that patients with asthma had a *H. pylori* seropositivity of 47.3% compared with the controls' rate of 38.1%. However, their study may be partially discredited because of the lack of peptic ulcer patients' data to eliminate the effects of confounding factors, which are known to affect *H. pylori* seropositivity. In addition, the study only compares the level of anti-*H. pylori* IgG. It should be remembered that in *H. pylori* the presence of CagA positive strains has been shown to be associated with interleukin-8 (IL-8) induction in gastric epithelium. The increase in the production of IL-8 may relate to the neutrophilic infiltration into the gastric epithelium, which is characteristic of *H. pylori* (Atherton et al. 1994; Sharma et al. 1995). It has been reported that CagA-bearing *H. pylori* strains are more virulent and cause more severe gastroduodenal diseases than CagA-negative strains (Maeda et al. 1998), so in our study we not only compare the level of anti-*H. pylori* IgG but also the level of anti-*H. pylori*-CagA IgG. According to our results, there was no significant difference in both anti-*H. pylori* IgG and anti-*H. pylori*-CagA IgG levels between the asthmatic and control subjects ($p > 0.05$), but the mean level for the peptic ulcer group was significantly higher than that of the asthmatic group ($p < 0.01$).

Despite the similarities in the pathogenesis of asthma and peptic ulcer disease, we did not find a relationship between asthma and *H. pylori*

infection. It seems that though the similarities between the pathogenesis in asthma and ulcerogenesis might exist, it is also possible that they are invoked by a respective independent mechanism. A previous epidemiological study, conducted by Kosunen et al. (2002) in a population of Finland, from 1973 to 1994 allergen-specific IgE prevalence rates and IgE antibody levels rose, and the increase occurred mainly in the subgroup with no antibody to *H. pylori*. They proposed the hypothesis that *H. pylori* infection may counteract atopic disease including asthma. If the hypothesis is correct, there will be a sound explanation for our results.

Since all the patients included in this study were non-smokers, the possible impact of cigarette smoking on both asthma and *H. pylori* infection should be regarded as a potential study limitation. Although *H. pylori* has been identified in the tracheobronchial aspirates in mechanically ventilated patients, neither identification in human bronchial tissue nor isolation from bronchoalveolar lavage (BAL) fluid have been achieved yet (Feldman et al. 1998). So to our knowledge, at present, the primary evidence for a link between *H. pylori* infection and respiratory diseases was based on case-control studies with relatively small numbers of patients. Future studies should be large enough for moderate-sized effects to be assessed. The pathogenetic mechanisms focused on the prevalence of CagA positive *H. pylori* strains and their induced proinflammatory markers also need further evaluation.

In conclusion, despite the sero-epidemiological association of *H. pylori* infection with many respiratory diseases, our data did not show such an association for mild asthmatic patients. Further studies should be undertaken to confirm the observed results.

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