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The Impact of *Helicobacter Pylori* Infection on Lung Function and Severity of Bronchial Hyperresponsiveness in Subjects with Allergic Asthma

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Abstract: Problem statement: There is evidence that *Helicobacter pylori* (*H. pylori*) infection may modify immune response and decrease the risk of asthma and other allergic diseases, as well. **Approach:** To assess the impact of *H. pylori* infection on lung function parameters and severity of Bronchial Hyperresponsiveness (BHR) in subjects with allergic asthma. A cross-sectional study including 38 *H. pylori* positive subjects with allergic asthma (18 males and 20 females, aged 21-54 years) and an equal number of *H. pylori* negative subjects with allergic asthma studied as a control was carried out. The impact of *H. pylori* infection was assessed by comparison of mean values of spirometric parameters and BHR severity (measured by histamine challenge and expressed as provocative concentration of histamine that causes FEV_1 fell by more than 20% of its base value-PC 20) between two examined groups. **Results:** The mean values of spirometric parameters were similar in both examined groups. The mean PC20 was lower in the group of subjects with allergic asthma and serological evidence of exposure to *H. pylori* but statistical significance was not reached (2.89 Vs. 3.14 mg mL^{-1}, $P > 0.05$). **Conclusion:** Our findings indicate that in cross-sectional analysis there is no significant relation between *H. pylori* seropositivity and the values of spirometric parameters and the degree of BHR severity in subjects with allergic asthma.

Key words: Allergic asthma, bronchial hyperresponsiveness, *Helicobacter pylori*, histamine challenge, spirometric parameters

INTRODUCTION

There is a strong evidence about the significant increase in the prevalence of allergic asthma and other allergic diseases in the last decades worldwide but the causes of this increase remain largely unknown. Suggested explanations include effects of air pollutants (e.g., diesel exhaust), changes in smoking habits, type of dwelling, family size (i.e., number of siblings), exposure to orofecal infections (Mutius and Sears, 2003; Matricardi *et al*., 2000; 2002; Salvi *et al*., 1999; Ball *et al*., 2000).

There is a recent evidence of inverse association between the *Helicobacter pylori* (H. pylori) infection and the frequency of allergic asthma and other allergic diseases (Blaser *et al*., 2008; D'Elios *et al*., 2009). H. *pylori* is a Gram-negative bacterium that chronically infects the stomach of more than 50% of the human population (varying from over 70% in developing countries to less than 40% in developed countries) and represents the major cause of gastroduodenal pathologies (Wotherspoon *et al*., 1991; Parsonnet *et al*., 1991). H. *pylori* gastric colonization is followed by mucosa infiltration of polymorphonuclear leukocytes, macrophages and T-helprer type 1 (Th1) lymphocytes with production of interleukin 12 (IL-12) and interferon-γ (IFN- γ) (D'Elios *et al*., 1997). On the other side, allergic asthma and other allergic diseases are orchestrated by T-helper type 2 (Th2) cytokines, such as IL-4 and IL-5 (i.e., Th 2 inflammation) (Barnes, 2003). This type of inflammation may be inhibited by IL-12 and IFN- γ, i.e., in subjects with allergic asthma

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the typical Th2 response can be redirected toward Th1 by the neutrophil-activating protein of H. *pylori* (Amedei *et al*., 2006; Codolo *et al*., 2008).

In addition, there is a evidence that H. *pylori* infection is associated with reduced growth in children and with consecutive negative impact on lung development (Perri *et al*., 1997; Hankinson *et al*., 1999). The chronic inflammatory response related to H. *pylori* infection is considered to be a potential risk factor for accelerated age-dependent decline in lung function which may result in lower lung function (Pavord *et al*., 2006).

To our knowledge, there is only few studies that investigated the association between exposure to H. *pylori* infection and objective measures of asthma and lung function. on lung function parameters and Severity of Bronchial Hyperresponsiveness (BHR) in subjects with allergic asthma. The present study aimed at investigation of the impact of H. *pylori* infection on lung function and BHR by comparison of spirometric parameters and degree of BHR severity (expressed as provocative concentration that causes FEV1 fell by more than 20% of its base value-PC 20) between a group of subjects with allergic asthma with serological evidence of exposure to H. *pylori* and a group of subjects with allergic asthma and negative serology for H. pylori.

METERIALS AND METHODS

Study design and setting: A cross-sectional survey was carried out in the Department of Cardiorespiratory Functional Diagnostics at the Institute for Occupational Health of R. Macedonia, Skopje-WHO Collaborating Center for Occupational Health and GA^{2}LEN Collaborating Center in the period May 2011-January 2012.

Study subjects: Fifty four subjects with positive serological finding for H. *pylori* infection and symptoms suggestive for asthma underwent diagnostic protocol for allergic asthma. Diagnostic criteria met 38 of them, 18 males and 20 females, aged 21-54 years.

In addition, an equal group of H. *pylori* negative subjects with allergic asthma matched by sex and age to H. *pylori* positive subjects with allergic asthma were studied as a control. All study subjects were informed about the study and their written consent was obtained.

H. *pylori* serological status: H. *pylori* serological status, i.e., quantitative detection of serum immunoglobuline G (IgG), was evaluated using the Siemens Immulite^R 1000 assay (a solid-phase, chemiluminiscent IgG assay) (Siemens, Germany). Seropositivity was considered in the case of finding of specific IgG concentration equal or more than 1 U/mL, while the subjects with serum concentration of specific IgG equal or less than 0.9 U mL^{-1} were considered as seronegative Immulite^R 1000 Chemiluminiscent Technology.

Allergic asthma diagnosis: According to the actual recommendations of the (Bateman *et al*., 2008), (GINA), a clinical diagnosis of allergic asthma was based on the presence of asthma symptoms (i.e., episodic breathlessness, wheezing, cough and chest tightness), one or more positive skin prick tests to common inhalant allergens, spirometry and on the positive histamine challenge (Dreborg and Frew, 1993).

Skin prick tests: Skin Prick Tests (SPT) to common inhalant allergens were performed in all subjects on the volar part of the forearm using commercial allergen extracts (Torlak, Serbia and Montenegro) of birch (5000 PNU), grass mixed (5000 PNU), plantain (5000 PNU), fungi mixed (4000 PNU), *Dermatophagoides pteronyssinus* (3000 PNU), dog hair (4000 PNU), cat fur (4000 PNU) and feathers mixed (4000 PNU). All tests included positive (1 mg mL^{-1} histamine) and negative (0.9% saline) controls. Prick tests were considered positive if the mean wheal diameter 20 min after allergen application was at least 3 mm larger than the size of the negative control (Dreborg and Frew, 1993; Bousquet *et al*., 2012). Atopy was defined as the presence of at least one positive SPT to common inhalant allergens (Frew, 2003).

Spirometry: Spirometry, including measures of Forced Vital Capacity (FVC), FEV_1, FEV_1/FVC ratio and maximal expiratory flow at 50, 25 and 25-75% of FVC (MEF_{50}, MEF_{25} and MEF_{25-75}, respectively), was performed in all subjects using spirometer PowerCube-Spiro for LF8 (Ganshorn Medizin Electronic GmbH, Germany) with recording the best result from three measurements the values of FEV_1 of which were within 5% of each other. The results of spirometry were expressed as percentages of the predicted values according to the actual recommendations of European Repsiratory Society (ERS) and American Thoracic Society (ATS) (Miller *et al*., 2005; Clausen *et al*., 1997).

Histamine challenge: The histamine challenge test was performed according to the actual ERS/ATS recommendations (Sterk *et al*., 1993; Crapo *et al*., 2000). Concentrations of 0.5, 1, 2, 4 and 8 mg mL^{-1} histamine (Torlak, Beograd) were prepared by dilution with buffered saline. The doses of aerosol generated by

Pari LC nebulizer with output rate 0.17 mL min^{-1} were inhaled by mouthpiece. Subjects inhaled increasing concentrations of histamine using a tidal breathing method until FEV_1 fell by more than 20% of its base value (provocative concentration 20-PC20). The test was considered positive if PC20 was equal or less than 4 mg mL^{-1} (Sterk *et al*., 1993; Crapo *et al*., 2000).

According to the ATS recommendations, BHR was categorized as moderate to severe BHR (PC20 < 1.0 mg mL^{-1}), mild BHR (PC20 = 1.0-4.0 mg mL^{-1}) and borderline BHR (PC20>4.0 mg mL^{-1}) (Crapo *et al*., 2000).

Statistical analysis: Continuous variables were expressed as mean values with Standard Deviation (SD), whereas the nominal variables as numbers and percentages. Analyses of the data involved testing the differences in prevalence and comparison of the means. Chi-square test (or Fisher's exact test where appropriate) was used for testing difference in the prevalence. Comparison of spirometric measurements and PC20 values was performed by independent-samples T-test. A P-value less than 0.05 was considered as statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 for Windows.

RESULTS

Demographic characteristics were similar in both H. *pylori* positive and negative subjects with allergic asthma (Table 1).

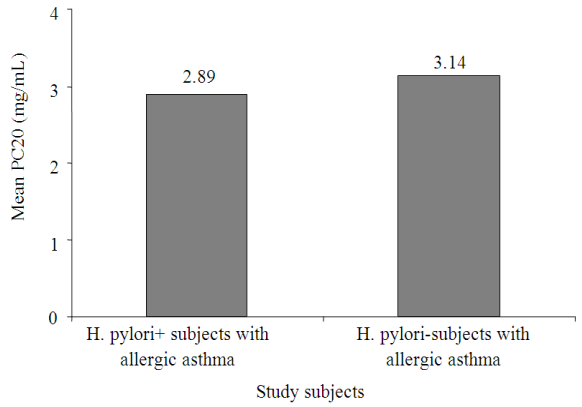

Fig. 1: Mean PC20 in H. pylori positive and H. pylori negative subjects with allergic asthma PC20: concentration of histamine causing a 20% fall in FEV1; H. pylori: *Helicobacter pylori*

Sensitization to common inhalant allergens in the study subjects is given on Table 2. Mite sensitization was the most important individual common allergen with no statistical difference between sensitized subjects in both examined groups (Table 2).

Table 1: Demographics of the study subjects

Common inhalant allergen	*H. pylori* positive subjects with allergic asthma (n = 38)	*H. pylori* negative subjects with allergic asthma (n = 38)
Males/females ratio	0.9	0.9
Age (years)	37.4±8.7	37.8±7.5
BMI (kg/m^2)	25.3±3.9	25.7±5.1
Mean IgG concentration (U/mg)	4.3±1.4	0.5±0.1
Family history of asthma	4 (10.5%)	3 (7.9%)
Smoking status		
Daily smokers	10 (26.3%)	12 (31.5%)
Smoking experience (years)	17.8±5.7	16.4±6.8
Cigarettes per day	15.9±6.1	14.8±5.9
Pack-years smoked	12.3±3.7	11.4±4.4
Ex-smokers	3 (7.9%)	4 (10.5%)
Passive smokers	11 (28.9%)	9 (23.7%)
Accompanying diseases		
Arterial hypertension	5 (13.2%)	3 (7.9%)
Diabetes mellitus type 2	2 (5.3%)	3 (7.9%)

Numerical data are expressed as mean value with standard deviation; frequencies as number and percentage of study subjects with certain variable. *H. pylori: Helicobacter pylori*; BMI: body mass index; kg: kilogram; m: meter; IgG: immunoglobulin G; U: unit; mg: miligram

Table 2: Sensitization to common inhalant allergens in both examined groups

Common inhalant allergen	*H. pylori* positive subjects with allergic asthma (n = 38)	*H. pylori* negative subjects with allergic asthma (n = 38)	P-value *
Birch	7 (18.4%)	5 (13.2%)	0.233
Grass mixed	14 (36.8%)	11 (28.9%)	0.129
Plantain	9 (23.7%)	7 (18.4%)	0.217
Fungi mixed	6 (15.8%)	7 (18.4%)	0.288
D. pteronyssinus	21 (55.3%)	23 (60.5%)	0.183
Dog hair	2 (5.3%)	3 (7.9%)	0.349
Cat fur	5 (13.2%)	4 (10.5%)	0.307
Feathers mixed	/	/	/

Data are expressed as number and percentage of the study subjects with certain variable. *H. pylori: Helicobacter pylori; D. pteronyssinus: Dermatophagoides pteronyssinus.* *Tested by chi-square test (or Fisher's exact test where appropriate)

Table 3: Mean values of spirometric parameters in both examined groups

Spirometric parameter	*H. pylori* positive subjects with allergic asthma (n = 38)	*H. pylori* negative subjects with allergic asthma (n = 38)	P-value*
FVC (%pred)	89.7 ± 10.8	88.4 ± 9.3	0.105
FEV_1 (%pred)	81.6 ± 7.9	82.8 ± 10.2	0.131
FEV_1/FVC%	77.8 ± 6.7	78.4 ± 8.1	0.226
MEF_{50} (%pred)	68.9 ± 16.3	66.5 ± 12.9	0.144
MEF_{25} (%pred)	63.2 ± 17.1	64.6 ± 14.8	0.162
MEF_{25-75} (%pred)	78.2 ± 15.8	76.4 ± 12.2	0.129

Data are expressed as mean value with standard deviation. *H. pylori: Helicobacter pylori*; FVC: forced vital capacity; FEV_1: forced expiratory volume in one sec; MEF_{50}, MEF_{25}, MEF_{25-75}: maximal expiratory flow at 50, 25 and 25-75% of FVC, respectively; % pred: % of predicted value. *Compared by independent-samples T-test.

We found similar mean values of spirometric parameters in both examined groups (Table 3).

We found lower BHR severity (expressed as a mean PC20) in H. *pylori* positive than in H. *pylori* negative subjects with allergic asthma, but the difference was statistically non-significant (2.89 mg mL^{-1} Vs 3.14 mg mL^{-1}, p = 0.117; independent-samples T-test) (Fig. 1).

DISCUSSION

According to the results of several studies *H. pylori* infection is associated with polarization of immune system with decreased risk for development of asthma and associated allergic disease (Cremonini and Gasbarrini, 2003; Shiotani *et al*., 2008). Results from the Bristol Helicobacter Project indicated that H. *pylori* infection is associated with substantially reduced risk for three atopic diseases (asthma, allergic rhinitis and atopic deramtitis) (McCune *et al*., 2003). However, results of some other studies did not find significant difference in levels of H. *pylori* antibodies in studies of atopy, asthma and wheezing (Jun *et al*., 2005; Cam *et al*., 2009).

On the other side, it has been suggested that exposure to *H. pylori* is associated with a decrease in lung function and may be a contributing risk factor for chronic Obstructive Pulmonary Disease (COPD) (Roussos *et al*., 2003). In the present study including *H. pylori* positive and negative subjects with allergic asthma we assessed the impact of exposure to *H. pylori* on lung function parameters and BHR severity. The examined groups included subjects with similar demographic characteristics. In either group there was a large proportion of daily and passive smokers, as well as a low proportion of ex-smokers, that is similar to its prevalence in R. Macedonia documented in our previous studies (Minov *et al*., 2006; 2008) and indicate still insufficient anti-smoking activities.

Mite sensitization and grass pollens were the most important common inhalant allergens in the subjects of both examined groups. Similar pattern of allergic sensitization was also registered in our previous studies on allergic asthma among adults in R. Macedonia (Minov *et al*., 2003; Karadzinska-Bislimovska *et al*., 1999).

Adult lung function is a consequence of the peak lung growth attained by the third decade and the subsequent rate of lung function decline occurring in the subsequent adult life (Sherill *et al*., 1989; Hole *et al*., 1996). As it was mentioned above *H. pylori* infection is associated with growth delay in children and has a negatively impact on lung development and lower lung function (Perri *et al*., 1997; Hankinson *et al*., 1999). In the present study we found similar mean values of FVC, FEV_1 and FEV_1/FVC ratio, as well as of MEF parameters, in both examined groups. Similar findings were reported by Fullerton *et al*. (2009) in their population-based study in adults that investigated impact of exposure to *H. pylori* on lung function, atopy and asthma. Namely, in cross-sectional analysis they found lower lung function as measured by FVC and FEV_1 but this association disappeared after statistical adjustment for either height or socio-economic status. In addition, longitudinally analysis indicated that *H. pylori* serological status had no effect on the decline in lung function over 9 years.

BHR, defined as an exaggerated response to the bronchoconstrictor (including pharmacological agents, non-isotonic aerosols, cold air, exercise, environmental allergens and occupational sensitizers) is one of the key features of asthma (Sterk and Bel, 1989; Joos and O'Connor, 2003). BHR is not specific only for asthma and may occur in the course of other diseases, such as COPD, allergic rhinitis, atopic dermatitis, cystic fibrosis and congestive heart failure (Bel and Chanez, 2003; Tashkin *et al*., 1992). The hyperreactivity of the airways in subjects with asthma is related to the degree of allergic inflammation, as measured by histamine or metacholine challenge (Barnes, 2003). In contrast to COPD, BHR in subjects with asthma is not related to the basic FEV_1 value and has higher severity (moderate to severe and mild BHR) which is related to blood eosinophil count and total serum Immunoglobuline E (IgE) (Josephs *et al*., 1990). In the study of (Fullerton *et al*., 2009), mentioned above the authors found similar prevalence of BHR (defined as a provocative dose causing FEV_1 fell by more than 20% of its base value-PD20) in subjects with positive and negative serology for *H. pylori* (Hole *et al*., 1996). In the present study we found lower degree of BHR severity in *H. pylori* positive than in *H. pylori* negative subjects with allergic asthma but the difference did not reach statistical significance.

The present study has some limitations. First, relatively small number of the subjects in the study groups could have certain implications on the data obtained and its interpretation. Second, the study design, i.e., cross-sectional analysis, could also have implications on the data obtained and its interpretation. Third, the serological data for exposure to *H. pylori* is unable to distinguish current from prior infection which limits interpretation of the associations observed. The strength of the study is the comparison of the degree of BHR severity between subjects with allergic asthma with positive and negative serology for *H. pylori* that, to our knowledge, so far has not been reported in published literature.

CONCLUSION

In conclusion, in a cross-sectional study including *H. pylori* positive and negative subjects with allergic asthma we found similar mean values of spirometric parameters, as well as similar degree of BHR severity, in both examined groups. Our findings support the need of further larger prospective studies in order to assess the complex relationship between *H. pylori* infection and development and manifestations of allergic asthma.

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