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ORIGINAL ARTICLE

Helicobacter pylori seropositivity protects against childhood asthma and inversely correlates to its clinical and functional severity

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Abstract
Background: In recent years, the prevalence of asthma has risen in developed countries, and its extent related to a change in our indigenous microbiota. *Helicobacter pylori* disappearance across the population represents a fundamental change in our human microbiota and has preceded the rise in asthma prevalence.
Objective: To assess the relationship between childhood asthma and *Helicobacter pylori* infection.
Methods: Quantitative determination of *Helicobacter pylori* IgG among 90 asthmatic children and 90 – age and gender – matched non-atopic, non-asthmatic healthy children was performed using ELISA in serum of all participants.
Results: Helicobacter pylori IgG seropositivity was found in 25.6% of asthmatics compared to 44.4% of controls. Asthmatics showed lower median *Helicobacter pylori* IgG titre compared to healthy controls. We also detected a significant inverse relationship between *Helicobacter pylori* IgG titre and asthma severity.
Conclusion: Helicobacter pylori seropositivity protects against childhood asthma and inversely correlates to its clinical and functional severity.

Introduction

There is wide geographical variation in the prevalence of asthma and allergic conditions world-wide, with substantial differences seen between low- and high-income countries,

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and between urban and rural communities.[1,2] Pre- and post-natal development of human immunity is remarkably continuous. The progressive immune response stabilisation at the sub-mucosal level during the first year of life arises from the interface between the host and their microflora.[3] As a part of the hygiene hypothesis, a protective role of *Helicobacter pylori* infection in the aetiology of asthma and allergic disease has been raised. This gained support from a range of epidemiological,[4–8] epigenetic,[9] and animal model studies.[10] Reduced risks of atopy and asthma have been reported in human studies,[4,11,12] both in children[4,11,13] and adults.[6,12] *H. pylori* is a bacterium which colonises the gastric mucosa of approximately half the world's population and is a main cause of peptic ulcer disease and gastric adenocarcinoma.[14,15] Its infection is usually established during early childhood, persists life long, and remains asymptomatic in over 85% of cases. However, prevalence of *H. pylori* infection in developed countries has been declining sharply for several years.[16] Additionally, the proportion of young children who got infected is extremely low nowadays, probably due to antibiotic use.[17] Also, increased antibiotic use in infancy has been suggested to limit exposure to gastrointestinal microbes and to predispose to asthma in later life.[18]

There is ample evidence that, both, exogenous microbes and endogenous microbial communities, human microbiota, shape the developing immune system and might be involved in the prevention of pathologic pro-inflammatory trails.[19] Exogenous infection and microbial substances including *H. pylori* infection may elicit a Th1-mediated immune response, which suppresses Th2 responses. Acquisition of *H. pylori* may be of importance in the induction of regulatory T cells, which could effectively reduce the possibility of allergic asthma.[20] Inverse associations between *H. pylori*, especially *cagA*+ strains, with asthma and related allergic disorders were reported, especially involving younger individuals, and with early life disease onset.[8,13]

Therefore, we conducted this study to assess frequency of *H. pylori* seropositivity among asthmatic children compared to healthy controls. The relation between *H. pylori* IgG seropositivity in asthma clinical and functional severity was also studied.

Methods

Study population

This cross-sectional study was performed during the period from March 2014 till December 2015. Ninety asthmatic children (52 boys, 38 girls), aged 1–17 years (mean ± SD; 7.05 ± 3.58 years), were randomly recruited from the Paediatric Chest Clinic, Children's Hospital, Ain Shams University. Patients were selected and their clinical severity was assessed according to the global initiative for asthma ''GINA, 2012''.[21]

Age (2–18 years, mean ± SD; 7.51 ± 4.19 years) and gender matched non-atopic, non-asthmatic healthy children ($n = 90$) were chosen as controls. They were selected from the geographic area surrounding the place of study. There was no significant difference in school grades between children participating in the study.

This study has complied with the principles laid down in the Declaration of Helsinki, adopted by the 18th World Medical Assembly, Helsinki, Finland, in June 1964, and recently amended at the 59th World Medical Assembly, Seoul, Korea, in October 2008. The entire protocol was approved by the institutional ethical committee. All parents or care givers provided signed informed consent for participation in the study as required.

Spirometry

All participants were examined and underwent spirometry. At least eight hours before the test, short-acting bronchodilators were stopped. Dynamic spirometry (Jaeger, Germany) was performed, with measurement of forced expiratory volume in 1st sec (FEV1) % of predicted, according to standards of both European Respiratory Society and American Thoracic Academy. The highest values of FEV1 of three forced expiratory manoeuvres were used. Severity of the disease was classified as mild/intermittent or more severe. PFT results classified as normal or obstructive disease, degree of disease control on treatment was classified to controlled or uncontrolled asthma.[21,22]

Sample collection and processing

Venous blood sample (5 ml) was withdrawn from each participant under complete aseptic conditions. Blood was placed into tubes without anticoagulant and left at room temperature for 30–60 min for spontaneous clotting. Serum was separated by centrifugation at 3000 rpm for 10 min. Serum samples were kept frozen at −80 °C until used in quantitative determination of *Helicobacter pylori* IgG according to the manufacturer's instructions of Serion ELISA Classic *Helicobacter pylori* IgG kit (Virion\Serion GmbH, Würzburg, Germany) and total IgE according to the manufacturer's instructions of IgE ELISA kit (General Biologicals Corporation (GBC), Taiwan).

Statistical analysis

Collected data were reviewed, coded, entered personal computer then analysed statistically by SPSS software version 15 (SPSS Inc., Chicago, IL, USA). Obtained data were presented as count and percentage for categorical variables. Data are presented as mean ± SD. The Mann Whitney *U* test was used to analyse differences between two groups. Comparison of three groups was performed using analysis of variance (ANOVA) and Fisher's protected least significant difference test or Chi-squared test. Kruskal–Wallis (χ^2) test was used for comparison of more than two groups in non-parametric variables. Correlations between data were analysed using Spearman's rank correlation test. Statistical significance was set at a value of $p < 0.05$.

Results

Clinical data of the asthmatic group are shown in Table 1. Symptoms suggestive of gastro-oesophageal reflux were significantly more frequent among asthmatics (25.55%) when

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