

Inverse Association Between *Helicobacter pylori* and Pediatric Asthma in a High-Prevalence Population

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Keywords

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Abstract

Background: *Helicobacter pylori*-associated disease has led to aggressive diagnostic and eradication protocols that are partially responsible for the decrease in prevalence of *H. pylori* carriage. Recent evidence indicates that in low-prevalence populations, *H. pylori* may have protective effects on allergic diseases. The aim of this study was to explore the relationship between pediatric asthma and *H. pylori* infection in a population with high prevalence of *H. pylori* infection.

Materials and Methods: A national referral laboratory was screened for all ¹³C urea breath tests performed in children aged 5–18 years between 2007 and 2008, for patient demographics and physician-diagnosed asthma. Data concerning asthma-associated medication usage were extracted from electronic medical records and databases. Data were analyzed using a stepwise logistic regression model.

Results: During the study period, 6959 patients underwent urea breath testing (average age 12.4 ± 3.5 years). Of these, 3175/6959 (45.6%) were positive for *H. pylori*, and 578/6959 (8.3%) had asthma. Rates of asthma in *H. pylori*-positive and *H. pylori*-negative children were 7.3 and 9.1%, respectively (odds ratio 0.82; 95% confidence interval (CI) 0.69–0.98; *p* = .032). We also confirmed that male gender, urban residence, and age are associated with childhood asthma.

Conclusions: We demonstrate an inverse association between *H. pylori* and pediatric asthma in a population with a high prevalence of *H. pylori*.

Helicobacter pylori has been associated with chronic gastritis, gastric and duodenal ulcers, gastric adenocarcinoma and MALT lymphoma, as well as chronic idiopathic thrombocytopenic purpura in adults, iron deficiency anemia, and other pathologies where the relationship either is less clear or does not necessitate treatment [1]. Furthermore, it has been declared a carcinogen by the World Health Organization [2]. In the decades that ensued, diagnostic tools and treatment protocols have been developed in an attempt to eradicate *H. pylori*.

Over a decade ago, attention was called to the interesting observation that parallel to the decrease in the prevalence of *H. pylori* and of *H. pylori*-associated diseases, there was an increase in other types of pathology

including esophageal adenocarcinoma [3,4]. While still only an association for which causality had not been proven, these were the first descriptions of potentially positive implications of *H. pylori*. In 2000, Matricardi et al. [5] demonstrated that asthma and atopy were less frequent in military cadets who had positive *H. pylori*, *Toxoplasma gondii*, or hepatitis A virus serologies. Since then, several groups have ignited a debate with their data which inversely associate allergic conditions with *H. pylori* infection including allergic rhinitis [6–8], asthma [6–9], eczema [6,8], food allergy [10], and more specifically with the pediatric onset of these diseases [7,11]. However, others have challenged the validity of these associations [12–17]. Thus, the aim of our study was to further delineate the association

between pediatric asthma and *H. pylori* infection with the largest pediatric cohort published to date. While previous studies utilized *H. pylori* serology as evidence of positivity, we compared data from pediatric patients who underwent ^{13}C urea breath testing (UBT), and studied the association between *H. pylori* infection and the presence of asthma based on UBT results and available demographic data.

Materials and Methods

This study was conducted using the database from the central *H. pylori* laboratory of Clalit Health Services (CHS), the largest health maintenance organization in Israel, with over 3.8 million insured members (53% of the total population) [18]. This laboratory performs all UBT studies for CHS. As the reliability of UBT results decreases below the age of 5 years because of technical difficulties performing the test in this age group, we limited our analysis to children aged 5–18 years.

Test results for the calendar years 2007–2008 were searched, and all tests with data available for age and gender were analyzed. Tests were reported positive by the laboratory when delta (δ) over baseline (DOB) ≥ 3.5 and negative when ≤ 3.5 . However, in light of the fact that the optimal cutoff value has not been adequately determined for children [19], we disregarded results between 3.5 and 5 to minimize the use of false-positive and false-negative results. When more than one test result was available for a particular patient during the study period, only the initial result was used in our analysis to diminish the influence of re-testing following bacterial eradication.

We used the electronic data warehouse of CHS to recover clinical data on the study group from January 2003 to September 2008. The CHS-computerized database contains patient-level information and includes electronic medical record (EMR) clinical data, as EMRs are available in all primary care clinics, as well as detailed demographic data from CHS registries. A diagnosis of asthma in the CHS chronic disease registry relies on recorded diagnoses from the EMR and hospital discharge diagnoses and is further enhanced using data on prescription of asthma-specific drugs. The content of the registry is validated periodically by the patient's primary physician.

For each child with an available UBT result, data were extracted including age, gender, rural vs. urban place of residence, area-level socioeconomic status and ethnicity, and an operational diagnosis of asthma within one month of the date that the UBT was performed. This limitation was used so as to minimize the inadvertent inclusion of children who actually had

alternative diagnoses such as bronchiolitis and viral-induced wheezing as infants which were misclassified in the past as asthma. Area-level data are generated by the average socioeconomic level and majority ethnicity of the primary care clinic at which the patient is registered. These clinic-level data are based on periodic reports released by the Israeli Central Bureau of Statistics of all Israeli localities which are then validated annually by clinic administrators.

^{13}C Urea Breath Test

This test utilizes the ability of *H. pylori*-produced urease to hydrolyze swallowed urea labeled with ^{13}C into carbon dioxide and ammonia. By comparing the baseline and test breath enrichments of exhaled CO_2 with the isotopic molecule, the DOB can be measured and the activity of the bacterial urease assessed.

Samples of exhaled air were collected from patients before ($\text{T0}'$) and 30 minutes after ($\text{T30}'$) each swallowed 75 mg of urea labeled with ^{13}C in 200 mL orange juice. The $^{13}\text{C}/^{12}\text{C}$ ratio was measured in a mass spectrometer (Analytical Precision AP2003, Northwich, UK). In this examination, the presence of ^{13}C indicates an enzymatic reaction of the ^{13}C urea with urease. Results are expressed as DOB ($\text{T30}'\text{-T0}'$).

Statistical Analysis

The association between the independent variables (including *H. pylori* test results) and existing asthma diagnosis was assessed using univariate analysis. The chi-square test was performed to identify associations between *H. pylori* and asthma in each category of variables: gender, ethnicity, residence, socioeconomic level, and age groups. Statistical comparisons between groups of scale variables such as age were performed using the unpaired Student's *t*-test. A stepwise logistic regression model was then constructed to assess the independent association of each variable with asthma, including all variables that had demonstrated a statistically significant effect in univariate analysis. Because *H. pylori* has been found to be correlated with socioeconomic status, we examined the Spearman correlation coefficient between these two variables. Because a correlation was identified, the logistic regression model was run both with and without socioeconomic status as a variable, and Nagelkerke R^2 was calculated. All statistical analyses were performed using the SPSS package for Windows (version 17.0.0; SPSS Inc., Chicago, IL, USA). A *p* value < 0.05 was considered significant. Confidence intervals are provided where appropriate.

The study received the approval of the institutional review board at Meir Medical Center, Kfar Sabba, Israel.

Results

During the study period, UBTs were performed in 6959 children aged between 5 and 18 years. The demographic characteristics are presented in Table 1. Of those tested, 45.6% (3175/6959) were positive for *H. pylori*, and 8.3% (578/6959) had a physician-entered medical diagnosis of asthma.

The prevalence of asthma in the *H. pylori*-positive and *H. pylori*-negative groups was 7.3% (233/3175) and 9.1% (345/3784), respectively (OR 0.79 95% CI: 0.66–0.94 $p = .007$). Additional variables that had a statistically significant association with asthma in univariate analysis included age, gender, area-level socioeconomic status, area-level ethnicity, and rural versus urban place of residence.

A stepwise multivariate regression model was comprised using the variables, and the results are presented in Table 2. These show that the key variables independently associated with asthma prevalence were *H. pylori* status (OR 0.82 95% CI 0.69–0.98), age (OR 0.92 95% CI 0.89–0.94 for each additional year of age), female gender (OR 0.74, 95% CI 0.62–0.89), and urban residence (OR 1.34, 95% CI 1.06–1.69). The Spearman correlation coefficient between *H. pylori* status and socioeconomic status was $r = -.167$ ($p < .001$), indicating a weak yet significant negative correlation. The logistic regression model was therefore performed both

Table 2 Variables significantly associated with asthma in children in multivariate analysis

Variable	B	Odds ratio	95% Confidence interval for odds ratio	<i>p</i>
<i>Hp</i> +	−0.196	0.82	0.69–0.98	0.032
Female gender	−0.297	0.74	0.62–0.89	0.001
Age (for each additional year)	−0.086	0.92	0.89–0.94	<0.001
Urban residence	0.293	1.34	1.06–1.69	0.014
Socioeconomic status	−0.122		0.88	NS
Ethnicity Israeli Arab	−0.021	0.98	0.63–1.51	NS

NS, nonsignificant.

Values in bold are statistically significant.

with and without the socioeconomic level, and the results were nearly identical (Nagelkerke R^2 with socioeconomic status = 0.027 and without 0.028).

Discussion

In this study of 6959 children who underwent UBT during 2007–2008, we show that *H. pylori* is an independent predictor for a positive diagnosis of pediatric asthma in a population with a high prevalence of *H. pylori* infection. To our knowledge, this is the largest study documenting such an association and the first to use pediatric UBT results rather than serologies that are considered less reliable in children [20]. In addition, our data support previous reports that female gender is protective while urban residence is an independent risk factor for pediatric asthma [21,22].

Our findings concur with previous reports which compared *H. pylori* seroprevalence with reported diagnoses of pediatric asthma [5,11]. These studies utilized *H. pylori* serology tests which have very broad ranges of both sensitivity and specificity (50–100%). This broad range could introduce considerable information bias in low-prevalence populations. Our utilization of a cohort that underwent UBT increased the reliability of the test results. Admittedly, UBTs are less reliable at very young ages [19]; we therefore set the minimum age for our analysis at 5 years and excluded borderline results. In addition, in this study, we did not rely on self-reported diagnoses of asthma; rather, a medical diagnosis was sought in the medical records, thus minimizing familial misrepresentation of recurrent viral respiratory diseases as asthma. Furthermore, the chronic disease registry data that we used are further enhanced using

Table 1 Patient demographic data

	<i>Hp</i> − <i>n</i> = 3784	<i>Hp</i> + <i>n</i> = 3175	<i>p</i>
Age (average ± SD)	12.4 ± 3.5	12.5 ± 3.5	NS
Male	34%	33.1%	NS
Ethnicity			
Israeli Arab	12.3%	22.9%	<.0001
Jewish Ultraorthodox	3.6%	4.3%	
General population	84.0%	72.8%	
Socioeconomic status			
Low	37.4%	53.1%	<.0001
Average	43.3%	36.0%	
High	19.3%	10.9%	
Residence			
Urban-Jewish	83.1%	74.8%	<.0001
Rural Jewish	5.5%	3.9%	
Non-Jewish	11.4%	21.3%	

NS, nonsignificant.

prescription data on asthma-specific drugs. In addition, significant ongoing organizational attention is dedicated to the validation process asthma diagnoses, as key quality measures are based on the accuracy of this diagnosis in the chronic disease registry.

The described negative association between pediatric asthma and *H. pylori* infection is intriguing because it may represent evidence of a mutual symbiosis of the bacterium and its host early in life, while, as Blaser [23] has previously theorized, in later life it acts as pathogen. The decreasing prevalence of *H. pylori* in the developed world, whether owing to improved sanitation, decreased crowding, antibiotic use, or the combination of these, may, in such a scenario, be contributing indirectly to the increase in asthma during the past several decades. This notion would also be supported by the posed "hygiene theory," which states that early exposure to pathogens shifts the cytokine balance toward a less atopic state [24]. Most studies seem to agree that the effect of *H. pylori* is more pronounced in children [7,11], indicating that the priming toward atopy is likely early phenomenon.

There have been several attempts to try to explain the influence of *H. pylori* in this regard. These focus on the ability of *H. pylori*, either in a pathogen-specific [25–27] or in a nonspecific [5,16] manner, to induce a cytokine profile (such as the T-helper(Th)1 response) that is less conducive to the development of atopy. In support of a pathogen-specific mechanism, some have shown that *H. pylori* is able to specifically induce a cytokine milieu that does not support atopy. Amedei et al. [25] found that addition of *H. pylori* neutrophil-activating protein to antigen-induced T-cell lines shifted the cytokine profile from a Th2 to Th1 profile. Th1 responses have also been described in vivo in *H. pylori*-infected gastric mucosa in general [26] and specifically in children [27]. In contrast, others have argued the *H. pylori* is part of a more general effect, which may be induced by multiple pathogens. Matricardi et al. [5] suggested that the route of transmission of a childhood-acquired pathogen (e.g. oral vs respiratory) was more important than the specific pathogen to drive the immune system toward a T-helper(Th)1 response which acts protectively against atopy. As such, *H. pylori* may also be a marker for the presence of other antigens that are often concomitantly present, owing to the inverse association of the bacterium and household hygiene. There is, however, no reason that each of these mechanisms needs to be mutually exclusive, and both may be functioning simultaneously, likely with an additive effect.

Conversely, Raj et al. [16] suggested that the finding of very low prevalence rates of *H. pylori* without a

concomitant increase in asthma in northeastern Peninsular Malaysia, a region where other enteric infections are still common, indicates that *H. pylori* may not be directly involved at all in the reduction in the odds of developing asthma. Rather, it may only be a marker for poor hygiene, which, through the hygiene theory, correlates well with a low prevalence of asthma.

An alternative explanation for the negative association is that *H. pylori* acts as a surrogate marker for antibiotic use. Children with asthma have been shown to receive more courses of antibiotics than nonasthmatic children [28]. As such, children with asthma may undergo low-level eradication of *H. pylori* during these excess episodes of antibiotic treatments when compared to nonasthmatics. This parameter could not be evaluated in the current study.

The high rate of *H. pylori* positivity found in our study population most likely stems from two etiologies. Firstly, Israel is a country in which the bacterium is highly prevalent. A survey performed in 1998 found approximately 40% infection rate in the 10- to 20-year-old age group [29], while a recent report by Kori et al. [30] found 32% positive stool samples in asymptomatic 1- to 5-year-old toddlers. In addition, the UBTs in this study were obtained from patients sent by physicians because of symptoms suggestive of *H. pylori* infection, and therefore, a rate of infection higher than in the general population is to be expected.

One may argue that a 1.8% decrease, while statistically significant, is not clinically relevant; however, the high prevalence of pediatric asthma [31] of approximately 9% in the United States may suggest otherwise. As the prevalence of the disease increases, the incremental differences translate into significant numbers of children potentially influenced. Regarding the increased risk of gastric adenocarcinoma in *H. pylori*-infected individuals, at this time there is no evidence to either support or contest that delaying treatment past adolescence in non-high-risk patients confers a higher risk of malignancy than treatment in childhood. However, the recurrence rate of infection in children is significant in high-prevalence societies [32], treatment protocols are becoming more complex, and antibiotic resistance has become a major issue in the eradication of *H. pylori* [33].

Methodological limitations might have contributed to biases in this study. The retrospective nature of this study and its reliance on patients having been sent by their primary physicians to perform UBTs for clinical indications rather than a strict population survey may have weakened the information obtained. Furthermore, the study design was unable to use a standardized diagnosis of asthma but rather depended on a diagnosis

provided by primary physicians. As mentioned, attempts were made to ascertain the diagnosis with available information on use of asthma medication and discharge diagnoses from hospitalizations and emergency department visits. The adequacy of specimen collections for UBT samples collected from many clinics throughout the country could not be established on a per-case basis; however, CHS uses quality assurance systems, as well as updated instructions which are sent out periodically from the central laboratory. In addition, the structure of the study did not allow for analysis of *cagA* status among the patients. Chen et al. [7], reported that the protective effect of *H. pylori* on asthma was largely due to those carrying *cagA*-positive strains. In that regard, *cagA* analysis would most likely strengthen the association we found. Similarly, we could not ascertain whether there were any differences in other microbial exposures, including both exogenous and endogenous flora which may also play a role in immune modulation. Finally, while our results demonstrate a negative association between *H. pylori* infection and pediatric asthma prevalence, the study was not designed to address the question of whether early eradication will increase the prevalence of pediatric asthma.

In conclusion, this study reaffirms, utilizing the combination of urea breath testing and the largest pediatric cohort to date, that *H. pylori* is an independent protective factor for a positive diagnosis of childhood asthma in addition to female gender, while urban residence is a risk factor. Prospective, long-term population-based studies, as well as immunological studies, will be able to further delineate the complex relationship between *H. pylori* and its hosts. The knowledge gained should trigger new discussion as it may have ramifications for public healthcare policy concerning the optimal timing of *H. pylori* eradication in the pediatric population.

Conflict of Interest

None to declare.

Funding

None to declare.

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