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The Association of Childhood Asthma and *Helicobacter pylori* Infection in Sardinia

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Background: It has been suggested that *Helicobacter pylori* infection might reduce the risk of atopic conditions, such as asthma, in childhood. This risk reduction could relate to the "hygiene hypothesis" which proposes an association between childhood exposure to microbes and risk of atopy.
Objectives: To examine the association between Hp infection and childhood acquired asthma in Sardinia.
Patients and Methods: Children from Northern Sardinia who were between the ages of 10 months to 6 years and were screened for Hp infection in 1994-1995 using IgG serology, were asked in 2012, whether they had developed asthma and/or allergic disease in pediatric age, using the global initiative on asthma guidelines questionnaire.
Results: A total of 64 children participated in the study. The sero-positivity for Hp infection was 14.1%. Eleven (17.2%) children had a confirmed diagnosis of asthma with onset before the age of 5 years, 85.9% were Hp negative and 14.1% Hp positive. Eight children of the 53 without asthma were Hp positive (15%) compare to one children positive for the infection of the 11 patients (0.09%) with asthma (8/53 vs. 1/11; P = 0.6). The majority of children (73%) were from urban areas and 43.8% had a family history of asthma or allergies. Multiple logistic regression analysis was not able to find a studied variable, including Hp infection, significantly associated with pediatric asthma.
Conclusions: Our results speak against Hp infection itself playing a role to protect from the risk to develop childhood asthma although household hygiene was not directly assessed.

Keywords: Child; Asthma; *Helicobacter pylori*

1. Background

Helicobacter pylori (*H. pylori*) is one of the most common chronic bacterial infections of mankind affecting approximately 50% of the world's population (1). The risk of acquiring *H. pylori* infection is related to socioeconomic status and living conditions early in life and is inversely related to household hygiene practices (1, 2). Once acquired, the bacterium becomes the dominant member of the gastric microbiota (3).

Asthma is the most common chronic disease in childhood in developed countries (4, 5). In Western nations, the rate of acquisition of *H. pylori* has declined whereas the incidence of childhood asthma has increased. For example, in the USA the prevalence of childhood asthma increased more than 50% between the 1980's and 1990's and by about 10% in the last decade (6). One hypothesis to explain this phenomenon is the so called "hygiene hypothesis" which links a reduced exposure to microbes in childhood with the increased incidence of atopy (7). This link is postulated to relate to the alterations in the balance between a Th1 and Th2 response to antigenic stimuli. Chen and Blaser (8) suggested that the inverse relationship between the increase in atopic diseases and fall in acquisition of *H. pylori* infections might be causal (i.e. *H. pylori* might occupy a metabolically active compartment in the stomach involved in the immunological barrier process that protects against allergic diseases). Alternately, *H. pylori* infection could be a surrogate marker for the level of household hygiene (i.e. general exposure to microbes).

2. Objectives

In this study we examined the association between *H. pylori* and childhood asthma in a cohort of children from Northern Sardinia.

3. Patients and Methods

Children from Northern Sardinia who were between the

Implication for health policy makers/practice/research/medical education:
It has been suggested that the inverse relationship between the increase in atopic diseases and fall in acquisition of *H. pylori* infections might be causal. Our study did not confirm any protective role for H. pylori infection in childhood asthma.

ages of 10 months to 6 years were screened for *H. pylori* infection in 1994 and 1995 using *H. pylori* IgG serology (9). A sample of venous blood was obtained from each child participating in the study in a clinical setting. The sample was a sample of convenience obtained by follow-up of a large cohort originally selected for a study of the prevalence and epidemiology of *H. pylori* infection among Sardinian children.

Serum was separated and frozen at -20°C until assay. The presence of IgG antibodies to the high molecular weight cell-associated proteins of *H. pylori* was measured using an enzyme-linked immunosorbent assay ELISA (HM-CAP, EPI, Westbury, NY). The specificity and positive predictive value of the HM-CAP ELISA were each previously shown to be 100%; the sensitivity is 98.7%, and the negative predictive value is 98.6%. There is no cross-reactivity with *Campylobacter jejuni*. The test has been validated in a pediatric context (10), and from the same geographic area (northern Sardinia) (9). *H. pylori* infection was defined as a positive ELISA result. Antibody titer above the cut-off value of 2.2 was considered positive.

The children were reinterviewed by telephone, using a validated questionnaire [the global initiative on asthma (GINA)] (11), whether they had developed asthma and/or allergic disease in pediatric age, in 2012. Only a definite diagnosis of asthma made by the pediatrician on the base of a careful process of history taking, physical examination, and eventually diagnostic studies using spirometry and the response to treatment according to GINA criteria was considered valid (11). The questionnaire was completed by the investigators with the cooperation of the parents of each child. Information obtained included the age and sex of the child, place of residence, occupation of the head of household, and exposure to animals (dogs and other animals, e.g. cats, parrots, pigs, goats, horses, ducks, donkeys, and chickens). Whether the child had a history of breast-feeding and the family history for respiratory diseases was also recorded. The socioeconomic status of the children was based on the occupation of the head of the household and recorded in 4 subgroups as follow: major professionals (persons who had graduated from university); minor professionals and administra-

Table 1. Study Variable Analyzed by Descriptive Statistics for Children With Asthma and Without Asthma

	Children	
	With Asthma, No. (%)	**Without Asthma, No. (%)**
	11 (17.2)	53 (82.8)
H. pylori		
Positive	1 (9.1)	8 (15.1%)
Negative	10 (90.9)	45 (84.9)
Gender		
Male	10 (90.9)	29 (54.7)
Female	1 (9.1)	24 (45.3)[a]
Contact with animals		
Yes	2 (18.2)	18 (34.0)
No	9 (81.8)	35 (66.0)
Family history of asthma		
Yes	7 (63.6)	21 (39.6)
No	4 (36)	32 (60.4)
Residence		
Urban	7 (63.6)	36 (67.9)
Rural	4 (36)	17 (32.1)
Socioeconomic Status		
High	7 (63.6)	36 (67.9)
Low	4 (36)	17 (32.1)
Day care attendance		
Yes	7 (63.6)	42 (79.2)
No	4 (36)	11 (20.8)

[a] P = 0.024 (Fisher exact test).

tors (persons who had not graduated from university); clerks and sales technicians; and semiskilled and unskilled workers, uneducated farmers, and shepherds. The results were assessed by a physician, and unclear answers were clarified by interviewing parents again.

Informed consent was obtained from children's parents for participation in the study, and the study protocol was approved by the Ethics Committee of the Faculty of Medicine at the University of Sassari (Sassari, Italy).

3.1. Statistical Analysis

Multiple logistic regression analysis, using the SPSS Enter procedure (SPSS statistical software, v. 16.0, Chicago, USA) was used to test the association between covariates and the probability to develop asthma. Odds ratios (ORs) and their 95% confidence interval (CI) were calculated as the relative amount of increase of odds from one-unit change in the independent variables, after controlling for the confounding effect of all covariates. For purpose analysis socioeconomic status was further categorized into "low" and "high" socioeconomic status. The residence variable was coded into a binary scoring system by giving "1" or "2" for urban or rural setting, respectively. The P lower than 0.05 was considered statistically significant.

4. Results

A total of 64 children (39 boys, 29 girls) from the original population of 150 (43%) participated in the study. Results are showed in Table 1. Eleven (17.2%) children had a confirmed diagnosis of asthma with onset before 5 years of age. Eight children of the 53 without asthma were *H. pylori* positive compare to one children positive for the infection of the 11 patients with asthma (8/53 vs. 1/11; P = 0.6), thus, not supporting the hypothesis of a "protective role" of *H. pylori* infection against asthma in children. Curiously this child was the only one who received breast-feeding. There were no significant associations observed between childhood asthma and the occupation of the head of the household. The history for contact with dogs was positive in only two children.

Multiple logistic regression analysis is reported in Table 2. There were no variables, including *H. pylori* infection, significantly associated with asthma. The covariate that influences mostly the OR was the female gender, although not significantly. Besides, the effect of gender was confirmed by the contingency analysis (male 10 and 1 female with asthma vs. male 29 and female 24 without asthma; P = 0.025). The negative number in the residence variable is associated to a greater risk to develop asthma for urban children.

5. Discussion

Our observations are consistent with the results of a recent meta-analysis by Wang et al. which showed that there was only a weak evidence for an inverse association between asthma and *H. pylori* infection (12). More specifically the Authors found a weak inverse association between *H. pylori* and asthma in cross-sectional studies with an OR of 0.84 and of 0.82 in the cohort studies. There were no significant inverse association between *H. pylori* and asthma in both case-control studies. In addition, stratifying by age in children and adults, significant inverse association between *H. pylori* and childhood asthma was not still observed by performing a quantitative meta-analysis (12). However there are studies reporting an inverse association between *H. pylori* infection and asthma in children under 10 years (8). Chen and Blaser in their cross-sectional analyses conducted using data from 7412 children, showed that *H. pylori* seropositivity was inversely associated with asthma in pediatric age, and the inverse association with onset of asthma before 5 years of age was stronger (OR : 0.58). *H. pylori* seropositivity also was inversely related to the recent onset of atopic disease (8). In a different study the same authors found that colo-

Table 2. Multiple Logistic Regression Analysis Was Used to Test the Association Between Covariates and the Probability to Develop Asthma

Covariates	Beta[a]	SE[a]	Significance	OR[a]	95% CI[a]for OR
H. pylori	-0.494	2.824	0.861	0.610	(0.002 – 154.609)
Gender	-1.851	1.113	0.086	0.157	(0.018 – 1.392)
Breast Feeding	-1.020	0.830	0.219	0.360	(0.071 – 1.834)
Animals	-0.558	0.935	0.551	0.573	(0.092 – 3.579)
Family history of asthma	-0.874	0.765	0.253	0.417	(0.093 – 1.867)
Residence	-0.368	0.792	0.643	0.692	(0.147 – 3.270)
Day care attendance	0.859	1.095	0.535	1.971	(0.231 – 16.846)
Socioeconomic Status	1.248	1.035	0.246	3.483	(0.424-28.642)
Constant	1.781	2.067	0.742	5.936	

[a] Abbreviations: Beta, regression coeficent; SE, standard error; OR, odd ratio; CI, confidence interval.

nization especially with *H. pylori* CagA positive strain was inversely associated with currently or ever having a diagnosis of allergic rhinitis, especially for childhood onset (OR : 0.55) (13).

In Jeddah, Saudi Arabia, 1432 children; were tested for *H. pylori* status by ELISA using IgG antibodies (HM-CAP; Enteric Products Incorporation, Westbury, NY). The prevalence did not differ according to nationality and gender but significantly increased with age in children with chronic asthma, chronic anemia and neurological impairment ($P < 0.01$ for all), length of illness, number of blood transfusions, number of hospital admission and type of feeding (14).

The issue regarding whether *H. pylori* infection itself vs. *H. pylori* infection acting as a surrogate marker for poor household hygiene has been tested in terms of assessing the prevalence of childhood asthma in an area where hygiene was poor but *H. pylori* infections were rare (15, 16). Such populations exist in Malaysia and Zanzibar and provide sites where the effects of hygiene and *H. pylori* can be separated (15-17). Studies in Malaysia have failed to confirm that any of the proposed dire consequences associated with the falling prevalence of *H. pylori* including an increase in childhood asthma, gastroesophageal reflux disease, or adenocarcinoma of the esophagus (15-17). If anything, the available studies in Malaysia cast doubt on any direct role for any protective role of *H. pylori* infection and are consistent with the hypothesis that the *H. pylori* infection instead represents a surrogate maker for poor household hygiene (18-21). The weakness of the current study is the fact that only a small cohort of children was investigated. The strength is that a follow-up was after seventeen years.

Conclusions: Our study did not confirm an association between lack of *H. pylori* infection and childhood asthma. However, we did not assess household hygiene directly but our results speak against the infection itself playing a role to protect from the risk to develop childhood asthma. The numbers of children from rural areas were also too small for us to examine whether the hygiene hypothesis itself was related to childhood asthma in this population. Prospective studies in Zanzibar and/or Malaysia are needed to be able to test whether the role of the hygiene hypothesis and whether *H. pylori* is involved, a surrogate marker of poor hygiene, or neither.

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Authors' Contribution

Study concept and design: David Y Graham and Maria P Dore. Data collection: Marco Massidda, Gian Franco Meloni, Sara Soro. Statistical analysis: Giovanni M Pes. Analysis and data interpretation: David Y Graham and Maria P Dore. Manuscript drafting: Maria P Dore and Gianni M Pes. Critical revision of the manuscript: David Y Graham.

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No relevant financial interests or financial conflicts within the past 5 years and for the foreseeable future.

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