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Helicobacter pylori Infection does not Protect Against Allergic Diseases: Evidence from a Pediatric Cohort in Northern Sardinia, Italy

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Abstract: Background: A decline in the prevalence of *Helicobacter pylori* infection has been claimed to increase the probability of developing atopic diseases. Aims: To evaluate the prevalence of atopic disorders in a pediatric population according to seropositivity against *H. pylori*. Methods: Children referred to the local Pediatric Hospital were investigated to identify risk factors, especially *H. pylori* infection, associated with atopic disorders. A questionnaire, including demographics, house size, history of breastfeeding, residence, school or daycare center attendance, exposure to animals, and a diagnosis of atopy, was filled out by a trained pediatrician according to parents' answers. A blood sample was collected from each participant. Results: Seroprevalence of *H. pylori* infection was 11.7% among 492 children (240 females); 32 of them had a diagnosis of asthma and 12 of allergy. No one child showed both conditions. Statistically significant differences in *H. pylori* seropositivity were not detected between children with or without atopy (8.4 vs. 12.6; p=0.233). Although atopic disorders were more frequent in children exposed to traditional risk factors none showed to be significant after adjusting for all covariates. Conclusions: *H. pylori* infection, serologically assessed, was not significantly associated with a reduced risk of atopic diseases in children.

Keywords: asthma; allergy; atopy; Helicobacter pylori; inflammation

1. Introduction

The term "hygiene hypothesis", postulated back in 1989 by David Strachan [1], refers to the observation that exposure to a reduced quantity of microbes in the excessively hygienic environments of modern times may raise the risk of developing atopic diseases including asthma. Although the original version of the hypothesis has been subject to serious criticism [2,3], more or less modified versions of it are still supported by some epidemiologists and considered capable of explaining peculiar aspects observed, especially in developing societies [4,5]. Although the precise mechanisms underpinning the ability of the microbic infection to protect against immune-mediated diseases have not yet been clearly elucidated, it is usually assumed that early exposure to microorganisms naturally colonizing an unsanitary environment might induce a maturation of the human immune system, making it resistant to the loss of immune tolerance later in life, thus triggering atopic, or more generally, immune-mediated diseases [6]. The role of specific environmental bacterial or parasitic species in the development of immune tolerance has been widely debated in the literature, and it is believed that it may extend to cover soil-transmitted helminthic [7,8], fungal [9] and even viral infections [10].

Among the various microorganisms, the bacterial load of which has progressively decreased in the environment due to the improved hygienic conditions, and whose infection has been advocated to explain the rise in allergic diseases, there is also the microaerophile *Helicobacter pylori* able to colonize the gastric mucosa [11-17]. The spread of this microorganism mainly occurs in environments characterized by poor hygienic conditions, such as those present in developing countries and in rural

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regions [18]. In such circumstances, children are infected at an early age, whereby their innate immune system is shunted in a way that maintains immune tolerance. If not pharmacologically eradicated, the infection persists for life in the stomach, owing to the establishment of a peculiar host-bacterial relationship based on a blunting of the host immune reaction, which becomes less efficient in clearing the invader. For this reason, it has been conjectured that the rapid improvement of the hygienic and sanitation conditions of several populations in the past decades may have drastically reduced the prevalence of *H. pylori* infection and, at least partially, decreased the alleged protection of the infection itself against immune-mediated diseases [1,19]. This hypothesis has been tested in numerous epidemiological studies, however, with conflicting results. Some studies found a significant protective effect [20-24] while a few others did not provide any evidence of such an effect [25,26]. Overall, the meta-analysis by Lionetti et al. in 2014 concluded that the weight of evidence is in favor of a protective effect [14]. Yet, in light of the criticisms recently addressed to the hygiene hypothesis in general, and the fact that the *H. pylori*-host relationship is not entirely coincident with that of other commensal microorganisms, a re-examination of the issue with the contribution of new data seems warranted.

Based on these premises, we aimed to evaluate the impact of *H. pylori* infection, assessed by serology, on the prevalence of atopic diseases in a cohort of children from Northern Sardinia, Italy.

2. Materials and Methods

2.1. Study design

Consecutive children referred for a visit to the Children's Hospital (University of Sassari, Italy) for any reason were screened for *H. pylori* infection by serology. A validated questionnaire [27] was completed by the pediatrician for each child in collaboration with their parents. The questionnaire was designed to collect information regarding age, sex, place of residence, number of persons living in the household, home size, history of breastfeeding, and attendance at daycare centers or school. In addition, data regarding exposure to animals, more specifically pets (dogs, cats, and parrots) or farm animals (pigs, goats, horses, ducks, donkeys, and chickens), were collected. At the enrollment, a sample of venous blood was collected, and the separated serum was stored at -20° C until processed. The main reason for an eligible child to be excluded from the study was the denial by the parents to give the informed consent.

2.2. H. pylori status

H. pylori status was assessed by using an ELISA test, as previously reported [28]. The test possesses a high specificity and positive and negative predictive value and was validated in children from the same geographic area. IgG titer above 0.21 was considered positive.

2.3. Allergy and Asthma

For the purpose of the study, information on the presence of asthma or allergic disease was recorded according to the parents' interview and double-checked by matching the treatment (antihistamine drugs, corticosteroids, β2-agonists).

2.4. Ethical considerations

Children were enrolled in the study after informed consent was obtained from their parents. The study protocol was approved by the Ethics Committee of the Faculty of Medicine at the University of Sassari (Sassari, Italy).

2.5. Statistical analysis

Continuous variables were expressed as the mean ± SD, while categorical variables were presented as the absolute and percent frequencies. An urban area was defined as a population center with more than 20,000 inhabitants, according to the local environmental administration. The house

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size was categorized according to the threshold of 100 m². Breastfeeding was stratified based on its duration reported by the mother, i.e., less than 6 months, between 6 and 11 months, and greater than or equal to 12 months. The ownership of animals took into account the distinction between pets and farm animals. School and daycare center attendance were considered separately. Body weight was divided into normal or reduced based on comparison with growth percentiles. A multivariable regression model was fitted, using atopic disease occurrence as a dependent variable and *H. pylori* status as an independent variable, adjusting for age, sex, residence, house size, breastfeeding, body weight, and animal ownership as covariates. The Hosmer-Lemeshow goodness-of-fit test was used to evaluate whether the regression assumptions were satisfied. Data were analyzed by the SPSS statistical software package version 20.0 (Chicago, IL, USA). Statistical significance was taken as $p < 0.05$.

3. Results

Characteristics of children participating in the study are illustrated in Table 1. Overall, a total of 492 children were recruited, aged from 7 months to ≥37 months, and 48.8% were female. Among the overall cohort, the seroprevalence of H. pylori was 11.8%, without any significant difference in the two sexes (9.2% vs. 14.3%). More specifically, the highest significant prevalence was detected among children older than 37 months (19.4%), living in rural areas (25.3%) and smaller houses (<100 mt2) (16.1%), breastfed for 6 to 11 months (26.7%), owners of animals, especially farms animals (33.3%) and attending daycare or school (15.3%). There were no significant differences in the body weight of the children with seropositivity for H. pylori infection compared with those without (Table 1). Among the studied children, atopic disorders, including asthma, were reported by their parents in 44 of them, corresponding to 8.9% of the total study cohort. Allergies were more common in children negative for H. pylori infection, according to serology, while the prevalence of asthma was slightly higher in seropositive children (12.5% vs. 87.5%) but without statistically significant difference (Table 1).

Table 1. Characteristics of 492 children according to *Helicobacter pylori* status assessed by serology.

Variables	Hp [1] IgG < 0.21 (n=434)	Hp IgG ≥ 0.21 (n=58)	P-value [2]
Age (months), n (%)			
7 – 12	58 (96.5)	3 (4.9)	**0.002**
13 – 18	73 (97.3)	2 (2.7)	
19 – 24	47 (88.7)	6 (11.3)	
25 – 30	46 (90.2)	5 (9.8)	
31 – 36	102 (86.4)	16 (13.6)	
≥ 37	108 (80.6)	26 (19.4)	
Sex, n (%)			
Female	218 (90.8)	22 (9.2)	0.078
Male	216 (85.7)	36 (14.3)	
Residence, n (%)			
Urban	369 (91.1)	36 (8.9)	**<0.0001**
Rural	65 (74.7)	22 (25.3)	
House size in m², n (%)			
≥ 100	226 (92.6)	18 (7.4)	**0.003**
< 100	208 (83.9)	40 (16.1)	
Breastfeeding, n (%)			
No	197 (89.5)	23 (10.5)	0.409
< 6 months	188 (90.8)	19 (9.2)	
6–11 months	33 (73.3)	12 (26.7)	

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≥ 12 months	16 (80.0)	4 (20.0)	
Ownership of animals, n (%)	358 (90.2)	39 (9.8)	**0.006**
No	76 (80.0)	19 (20.0)	
Pets	16 (66.7)	8 (33.3)	
Farm animals			
School, n (%)			
No	271 (93.2)	49 (6.8)	**0.011**
Day care center	96 (84.7)	7 (15.3)	
School	67 (97.1)	2 (2.9)	
Weight, n (%)			
Normal	343 (87.1)	51 (12.9)	0.111
Reduced	91 (92.9)	7 (7.1)	
Allergy and/or asthma, n (%)	397 (88.6)	51 (11.4)	0.374
No	9 (75.0)	3 (25.0)	
Allergy	28 (87.5)	4 (12.5)	
Asthma	0 (0.0)	0 (0.0)	
Allergy and asthma			

[1] *Helicobacter pylori*; [2] Values in bold are statistically significant.

The Figure 1 reports the rates of atopic disorders according to the age ranges, showing that, especially asthma, occurred beyond 13 months of age. Although asthma has been reported to be more commonly present in individuals with other atopic diseases, such as atopic dermatitis and allergic rhinitis [29], no one studied participant displayed a double condition in our study (Table 1).

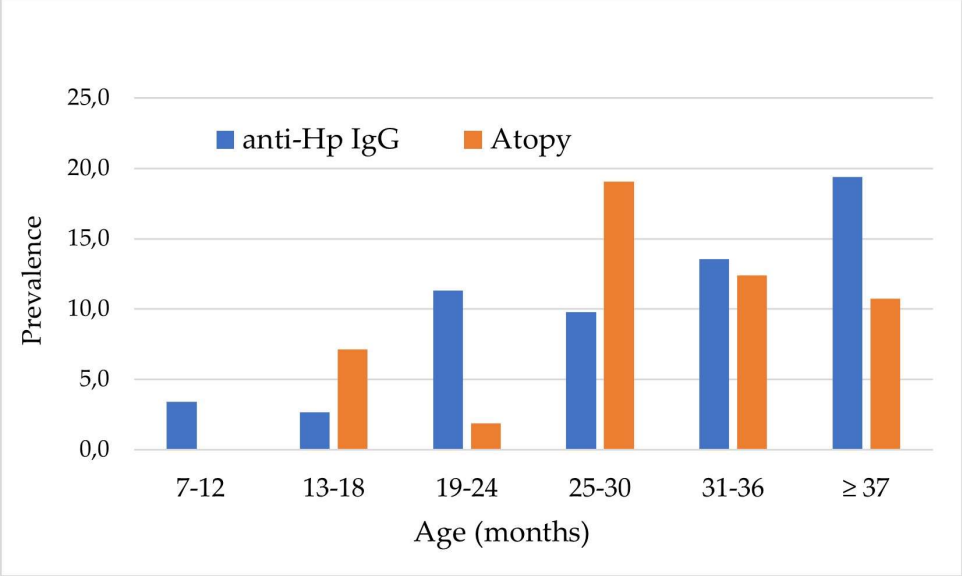

Figure 1. Prevalence distribution of seropositivity for *H. pylori* infection and atopy disorders in 492 children according to age ranges.

Table 2 listed the atopy rates in relation to the covariates included in the study. Although atopy was more frequent in oldest and male children living in urban areas, small houses, exposed to animals, and attending school, statistically significant differences were observed only for the oldest

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age and breastfeeding practice. More importantly, significant differences in the frequency of atopy between *H. pylori* seropositive and seronegative children were not observed.

Table 2. Baseline characteristics of 492 children according to atopy.

Variables	No atopy (n=448)	Atopy (n=44)	p-value *
Age (months), n (%)			
7 – 12	59 (100.0)	2 (3.3)	
13 – 18	70 (93.3)	5 (6.7)	
19 – 24	51 (96.2)	2 (3.8)	**0.001**
25 – 30	42 (82.4)	9 (17.6)	
31 – 36	105 (89.0)	13 (11.0)	
≥ 37	121 (89.0)	13 (9.7)	
Sex, n (%)			
Female	222 (92.5)	18 (7.5)	0.420
Male	226 (89.7)	26 (10.3)	
Residence, n (%)			
Urban	368 (90.9)	37 (9.1)	0.482
Rural	80 (92.0)	7 (8.0)	
House size, n (%)			
< 100	215 (90.0)	29 (11.9)	0.406
≥ 100	233 (92.1)	15 (6.0)	
Breastfeeding, n (%)			
No	197 (89.5)	23 (10.5)	**0.025**
Yes	251 (92.3)	21 (7.7)	
Ownership of animals, n (%)	361 (90.9)	36 (9.1)	0.827
No	87 (91.6)	8 (8.4)	
Yes			
School, n (%)			
No	297 (92.8)	23 (7.2)	0.063
Yes	151 (87.8)	21 (12.2)	
H. pylori IgG			
< 0.21	398 (91.7)	36 (8.3)	0.233
≥ 0.21	50 (86.2)	8 (13.8)	

* Values in bold are statistically significant.

After adjusting for all covariates (Table 3), not one traditional risk factor was found to be statistically associated with asthma or atopy, including *H. pylori* infection.

Table 3. Odds ratios (ORs) and their 95% Confidence Interval (CI) for atopic disease in 492 children older than 6 months.

Variables, n (%)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
Age		

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< 24 months	reference	reference
≥ 24 months	1.70 (0.88–3.30)	1.10 (0.41–2.94)
Sex		
Female	reference	reference
Male	1.29 (0.69–2.41)	2.17 (0.95–4.44)
Residence, n (%)		
Urban	reference	reference
Rural	0.73 (0.30–1.78)	1.46 (0.64–3.35)
Breastfeeding, n (%)		
No	reference	reference
Yes	1.07 (1.57–2.00) *	1.91 (0.75–4.84)
Ownership of animals		
No	reference	reference
Yes	2.29 (1.26–4.19)	0.65 (0.23–1.86)
School, n (%)		
No	reference	reference
Yes	1.80 (0.96–3.35)	1.58 (0.57–4.39)
Helicobacter pylori status		
negative	reference	reference
positive	1.81 (0.80–4.11)	0.79(0.22–2.84)

* p-value statistically significant.

4. Discussion

The results obtained in this study do not either confirm an inverse or a positive relation between atopic disorders, including asthma and *H. pylori* infection in children. These findings are consistent with our previous study conducted in the same geographic area [30]. In children aged 10 months to six years, screened for *H. pylori* infection using IgG serology and followed up for the occurrence of asthma and or allergic disease for seven years. 17.2% developed asthma, according to the Global Initiative on Asthma (GINA) [31], before the age of five years. Among them, 85.9% were *H. pylori* negative and 14.1% *Hp* positive [30]. By multiple logistic regression analysis *H. pylori* infection did not result significantly associated with childhood asthma.

Interestingly, we observed an overall reduction in the prevalence of *H. pylori* infection compared to previously reported data in a cohort of school children (22% vs. 11.7%) [27]. A decline in *H. pylori* infection has been observed in the last decades across most high-resource countries [32]. However, it cannot be ruled out that the increased use of antibiotics in the pediatric age [33], for which Italy holds the record in Europe, and the ensuing accidental eradication of the bacterium have contributed, at least in part, to the reduction in prevalence.

The traditional risk factors implicated in the acquisition of *H. pylori* infection were also confirmed in this study. Previously, in the same geographic area, the prevalence of *H. pylori* infection in 2810 school children was significantly higher among children living in rural areas compared with those living in urban areas (OR 3.8; 95%CI 3.2–4.7), and the risk was even greater for children who had dogs (OR, 1.8; 95%CI, 1.3–2.6) [27]. Ownership of animals, especially farm animals, and crowding (living in small-sized apartments/houses) were confirmed as risk factors significantly associated with *H. pylori* seropositivity also in the present study. Attendance at daycare centers and breastfeeding did not show a lower association with seropositivity [27,34,35].

Asthma is a chronic inflammatory disease of the airways, characterized by obstruction of the bronchial tree caused by inflammation of the lower airways and its consequences, generally

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reversible. The condition is the result of complex interactions between multiple risk factors and genetic influences that vary according to age, sex, breastfeeding, residence, exposure to animals, and school attendance, among others. Asthma represents the most frequent chronic respiratory disease in childhood, with a general prevalence in Italy estimated at around 10% (https://www.salute.gov.it/), not different from the prevalence found in our cohort (8.9%) with a tendency to be higher after 18 months of age.

Although childhood asthma has been reported to predominate in male children for a number of reasons, overall explained by sexual dimorphism in lung physiology [36,37], in our study, a statistically significant difference was not detected between sexes. However, similarly to our findings, some studies concluded that there are no sex differences in asthma severity [38,39]. Breastfeeding was repeatedly reported as a protective factor against asthma by several researchers [40-44]. This is understandable; breastfeeding helps to prevent respiratory tract infections [41]. Furthermore, some oligosaccharides contained in human milk, involved in specific (secretory immunoglobulin A, immunoglobulin M, and G) and nonspecific immunity (lactoferrin) and glycolipids [42], are able to reduce the virulence of pathogens and to modulate the immune response, thus attenuating the damage to the bronchial tree [42]. In addition, evidence has indicated that breastfeeding may help protect lung function in individuals exposed to high levels of air pollution, including secondhand smoke [43,44], suggesting the role of human milk in lung development. According to the literature, a lower prevalence of asthma was also found in our breastfed children.

In agreement with our results, early exposure in life to pets and or farm animals has been reported to be protective against the development of asthma and allergic disease [45,46]. Based on the rationale of greater allergen exposure, in adjusted analyses, the risk of asthma and allergen sensitization were increased in children living in urban areas compared to children living in rural areas [47]. The authors were able to demonstrate that the composition of airway and gut microbiotas differed between children living in urban and rural areas and suggested that changes in the child microbiota associated with the urban environment may increase the risk of asthma and atopic disorders, probably via cross-talk with the developing immune system [47]. However, although age, female sex, urban residence, breastfeeding, animal ownership, and no school attendance showed an inverse association with asthma and atopic disorders, as expected, none of these risk factors showed to be statistically significant predictors after adjusting for all covariates. More importantly, *H. pylori* infection was not found to be significantly associated with pediatric asthma.

H. pylori infection is considered a proxy of poor hygiene condition, and it has been supposed that, in the past century, improvements in household amenities, higher standards of personal hygiene, and reduced family size have decreased the opportunity for cross-infection in young families, and exposure to different antigens favoring earlier expression of atopic disease. However, in countries such as Malaysia and Zanzibar, with poor hygiene conditions and a very low prevalence of *H. pylori* infection, an increase in childhood asthma was not observed [48-51]. Several reports confirm an increase in asthma and atopic disorders, especially in high-income countries [52]. According to that, some studies have reported a lower prevalence of *H. pylori* infection in asthmatic patients, confirmed by a meta-analysis including 106 articles for a total of 28,283 patients [53] (OR= 0.84, 95%CI: 0.73–0.96, P = 0.013). However, a subgroup analysis indicated a similar prevalence in the asthmatic group and the control group of CagA-positive *H. pylori* infection [53]. However, other studies have reported neutral results or even a positive association between infection and asthma [30,54]. These contrasting results may be attributed to the different *H. pylori* detection methods used in the studies.

Some molecular aspects of the *H. pylori*-host interaction may help explain our results. Unlike other microorganisms, the bacterium does not favor the proper maturation of the immune system but, on the contrary, induces a tolerogenic phenotype in the dendritic cells, able to facilitate the persistence of the infection [55]. These tolerogenic cells, through interleukin-10 release, could inhibit the development of effector T cells, thereby creating an imbalance that induces later the appearance of exaggerated responses in the host [56].

The present study has several limitations that deserve discussion. Most data were self-reported by parents who may have exaggerated the importance of some environmental factors. Given that the study was limited to a pediatric population in Northern Sardinia, it may in some way have limited the generalizability of the results obtained, and finally, the cross-sectional design made us unable to establish causality.

5. Conclusions

In conclusion, in our study, the *H. pylori* infection, assessed through serological tests, did not significantly reduce the risk of allergy and atopy in children, suggesting that this bacterium—unlike other microorganisms—does not protect against atopic diseases by altering the host immune response.

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Informed Consent Statement: Informed consent was obtained from the legal tutors of all subjects involved in the study.

Data Availability Statement: Data supporting reported results can be available upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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