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The Relationship between *Helicobacter pylori* (*H. pylori*) and Atopy and Allergic Diseases

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Abstract

Reduced exposures to gastrointestinal microbiota have been proposed as an explanation for the increase in the prevalence of allergic diseases. The aim of this research is to study the relationship between *H. pylori* and atopy/allergic diseases. 210 children (aged 2-16 years old) with or without allergic diseases were selected from the Asthma Clinic in Al-Karak Governmental Hospital to participate in the study. Their parents were asked to fill out questionnaires. An Atopy test was done and the serum IgG antibodies to the *H. pylori* antigens were measured. Out of the 210 children, 38 were *H. pylori*-positive patients (18.1%), 31 cases (14.1 %) were atopic, and 60 patients (28.6%) were asthmatic. Out of the 38 *H. pylori* positive patients, only one case (2.6 %) was atopic. A significant negative association between *H pylori* and asthma was observed in the less than nine-year-old age group (p value = 0.007). The negative association between *H. pylori* and atopy was significant in males (p value = 0.02).The factors that best predict asthma include a family history of asthma, positive *H. pylori*, positive allergy test (p values of <0.005). Our findings showed that there is an inverse correlation between *H. pylori* and atopy.

Keywords*: Helicobacter pylori*; Asthma; Childhood Asthma, Jordan

1. Introduction

It is now known that allergic diseases develop from the interaction between the host immune system and some environmental factors (Rigoli *et al.*, 2011). The high geographical variability in the prevalence of these diseases suggests a decisive impact of the environmental factors (such as the geo-climatic and the socioeconomic ones) on their pathogenesis. Indeed, allergies are more common in the northern hemisphere, as well as in developed countries more than in developing ones (Mallol *et al.*, 2013). The characteristic epidemiological trend of atopic diseases seems to be also linked to the environmental changes that had occurred over the last decades in industrialized countries including the increase in outdoor and indoor pollution (combustion of fossil fuel, high volume of traffic, biomass combustion products, tobacco smoke), climatic changes (warmer temperature that causes early springs), and the improvement of hygienic conditions (changes in exposure to microbiota).

H. pylori, gram-negative, microaerophilic gastric bacteria persistently colonize much of the world's population. Whereas nearly all adults are *H. pylori*-positive in developing countries, with socioeconomic development, prevalence has decreased substantially (Dooley *et al.*, 1989). *H. pylori* is almost exclusively acquired in childhood (Blaser and Atherton, 2004), and the antibody responses are present for decades or for life, and are consistent with the persistent gastric colonization. *H. pylori* virulence is affected by the presence of the 35-40-kb cag pathogenicity island that can be detected by the identification of the CagA gene or its product (CagA). *H. pylori* colonization induces a continuous gastric inflammation, which is more pronounced with CagA+ strains (Suerbaum and Michetti, 2002), and leads up to a diminished gastric acidity (Kuipers *et al.*, 1995). Serologic assays to detect antibodies to the CagA protein enhance the overall detection of *H. pylori*; in particular the detection of the more interactive (CagA+) organisms.

As the hygiene hypothesis confirms, the most important factor associated with the large spreading of the atopic disease is the decreased exposure to food-born and oral-fecal infections, including the *Helicobacter pylori* (*H. pylori*) infection, as a result of improvements in the hygienic conditions in developed countries (Matsushima and Nagai, 2012). There is some evidence of an inverse association between atopy/allergic diseases and the *H.*

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pylori infection, although further studies are still needed (Reibman *et al.*, 2008; Daugule *et al.*, 2015).

Despite the fact that these clinical studies confirm the inverse association between *H. pylori* and asthma, some studies reported different results regarding asthma, other allergic diseases, and in general atopy. On the other hand, many researchers denied the hygiene hypothesis, considering gastrointestinal infections as triggers of allergic diseases, rather than protective factors because of their capability in increasing the mucosal permeability. Thereby asserting that these infections could have facilitated the penetration of allergens and the loss of oral tolerance (Jun *et al.*, 2005; Baccioglu *et al.*, 2008; Raj *et al.*, 2009; Lee *et al.*, 2015). Moreover, a recent study points that *the H. pylori* infection acts most likely as a good marker for the presence of poor hygiene (Miftahussurur *et al.*, 2017).

The aim of this article was to conduct a cross-sectional study on the relationship between *H. pylori* and atopy/allergic diseases to investigate whether or not there is negative association between *H. pylori* and atopy or allergic diseases among children in the area.

2. Patients and Methods

2.1. Study Design

The association between atopy/allergic diseases and the *H. pylori* infection is the focus of the current research investigating the *H. pylori* seroprevalence in atopic patients, those with allergic diseases, and in healthy individuals. Atopy is defined through the skin prick test reactivity to a panel of aeroallergens (olive pollen, mixed-grass pollen, dog dander, cat dander, Dermatophagoides Farinae, Dermatophagoides pteronyssinus, Cereals, Compositae, salsolakali and Wallpollitory). Allergic diseases are defined with the presence of a diagnosis of asthma, self- reported allergic rhinitis (Hey fever), self-reported atopic dermatitis, self- reported food allergy, or with the presence of allergic manifestations (such as pruritus of skin or eyes, nasal discharge, coughing and dyspnea) determined through questionnaires. The *H. pylori* infection status will be tested by serum anti *H. pylori* IgG and anti-CagA antibodies.

Several cross-sectional studies have investigated the relationship between the *H. pylori infection* and allergic diseases. Most of them have been conducted in developed countries, where atopy is more common, and the decreasing trend of the *H. pylori infection* is effective. This study will be the first of its kind to be done in Jordan. It was approved by the Ethics and Scientific Committees of the Faculty of Medicine at Mutah University. It was conducted between October, 2016 and June, 2017 at Al-Karak Governate Hospital in Al-Karak, Jordan. After signing the consent forms by the parents, 210 child patients with/without atopy or allergic diseases (cases) filled out questionnaires, and then two tests were done for all of them, namely the skin prick test (done in the Child Respiratory Clinic of Al- Karak Governmental Hospital), and the *H. pylori* test (done in a private laboratory) . The questionnaire included questions related to the demographic characteristics (age, sex, and residence), socio-economic status (education level, the monthly family income), and clinical characteristics (history of asthma, allergic rhinitis (Hey fever), atopic dermatitis, food allergy, family history of allergic diseases, and history of any *H. pylori* related- gastro-intestinal (GI) symptoms as dyspepsia regurgitation heart burn, anemia, poor growth, and any use of anti-acids). The body mass index (BMI) was calculated for all the participants.

The Odds Ratios (OR) of atopy and allergic diseases associated with the presence of *H. pylori* will be estimated. The effect of other factors such as age and sex will be analyzed as well. If the observed association is significant, more insights into the underlying mechanisms could provide clues to the possible therapeutic opportunities of allergic diseases.

2.2. Serum Antibody Analysis

Serum anti-*H. pylori* IgG antibody levels were determined in patient serum using a one-step *H pylori* test device from ABON (Herbrink and Van Doorn, 2000; Alsaimary *et al.*, 2014). Blood samples were collected from the children. The blood was either allowed to be clotted and the serum separated, or centrifuged (400xg for 5 mins.) to separate the serum. Then the serum was immediately examined by the rapid diagnostic *H. pylori* kit–Abon Biopharm (Hangzhou) Co., Ltd. P.R. China. The one- step *H. pylori* test device (serum/plasma) is a qualitative membrane based immunoassay for the detection of *H. pylori* antibodies in serum or plasma. In this test procedure, the anti-human IgG is immobilized in the test line region of the test. After the specimen was added to the specimen well of the device, it reacted with the *H. pylori* antigen coated particles in the test. This mixture migrates chromatographically along the length of the test, and interacts with immobilized anti-human IgG.

2.3. Statistical Analysis

Categorical variables were analyzed using the chi-square test or Fisher's exact test. Basically, the chi-square test was used in this study. However, Fisher's exact test was used instead of chi-square test when >20% of the expected frequencies were five or less. The binary logistic regression model was used to analyze dichotomous variables according to predictor variables. Independent variables with p values <0.20 in univariate analyses, and those already known to be strongly associated with the outcome variable were examined in multiple logistic regression models. A p-value <0.05 was considered significant. All p values are presented without correction for multiple testing. All analyses were performed with the Statistical Package for the Social Sciences, (ver. 16.0) for Windows software (SPSS Inc., Chicago, IL).

3. Results

3.1. Characteristics of the Study Groups

The youngest child examined in this study was two years old, while the oldest was sixteen years old. One-hundred and twenty-eight children (61%) were males and 82 (39 %) were females. The mean age was 8. 7 ± 3.4. Thirty eight of the cases (18.1) had a positive *H. pylori* test (male=17.9, female=18.3). Children with *H. pylori* had a mean age of 9.4 ± 3.1, while those without this infection had a mean age of 8.6 ± 3.5; no significant difference was

observed. Thirty one of the cases (14.8) had positive allergy test (Skin Prick Test). The mean age of children with atopy was 7.6 ± 4.3, while the mean age of those without atopy was 8.9 ± 3.2; no significant difference was indicated. The Mann Whitney U test was used to compare the mean age of children. Sixty of the cases (28.6%) had doctor diagnosed- asthma. The percentages of the self-reported allergic rhinitis (Hey fever), self- reported atopic dermatitis, self- reported food allergy were 48.1, 19, 26.2, respectively (Table 1).

Table 1. Characteristics of the study population.

Variable	Mean± SD	Number	Percentage (210=100%)
Weight (Kg)	38.2±1.7		
Height (cm)	134±220		
BMI	20.8±1.1		
Age (Years)	8.7±3.4		
Gender			
Male		128	61
Female		82	39
H pylori test			
Positive		38	18.1
Negative		172	81.9
Atopy			
Positive		31	14.8
Negative		179	85.2
Doctor diagnosed asthma			
Positive		60	28.6
Negative		150	71.4
Self-reported hay fever			
Positive		101	48.1
Negative		109	51.9
Self-reported eczema			
Positive		40	19
Negative		170	81
Self- reported food allergy			
Positive		55	26.2
Negative		155	73.8
Family history of asthma			
Positive		70	33.3
Negative		140	66.7
Family history of hay fever			
Positive		76	36.2
Negative		134	63.8
Family history of food allergy			
Positive		38	18.1
Negative		172	81.9
Family history of eczema			
Positive		50	23.8
Negative		160	76.2

3.2. H. pylori Status and Atopy

The skin prick test was done for all the child participants. The percentage of atopy in the whole data was 14.8. Similar findings were also obtained from an international study (Asher *et al*., 1995). The aero-allergens used in the test were olive pollen, mixed-grass pollen, dog dander, cat dander, Dermatophagoides Farinae, Dermatophagoides pteronyssinus, Cereals, Compositae, salsolakali and Wallpollitory; there sensitization percentages are presented in Figure 1.

The *H. pylori* test was done for all of the study population. The prevalence of seropositivity was 18.1.The *H. pylori*-related gastrointestinal symptoms were recorded based on the questionnaires provided. The percentages of these symptoms in both seropositive and seronegative patients were shown in Figure 2 (*p* value <0.05).

Out of the 38 *H. pylori*-positive patients, one case (2.6 %) was atopic, while out of the 172 *H. pylori* negative patients 30 cases (97.4%) were non-atopic, with the difference being statistically significant (*p* Value = 0.02) (Table 2, Figure 3).

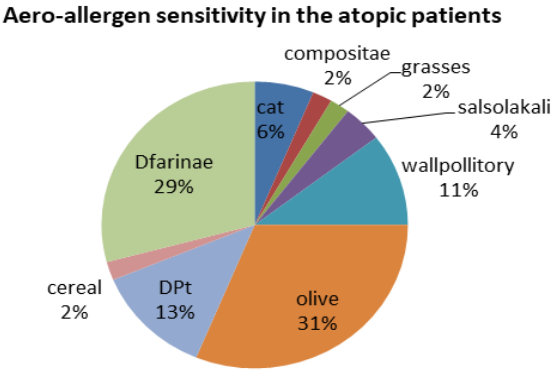

Figure 1. Percentages of aero-allergen sensitivity in the atopic patients

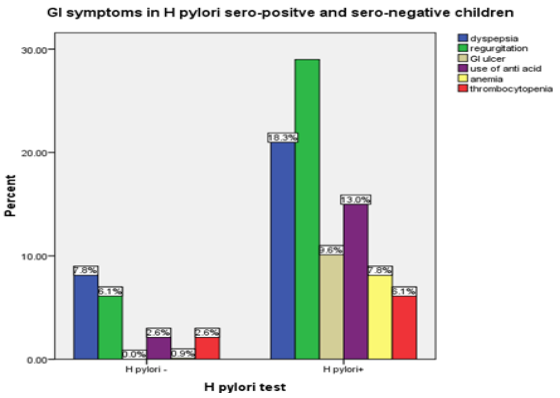

Figure 2. Prevalence of *H pylori*- related symptoms in sero-positive and sero- negative children**.**

Table 2. Frequency of distribution of allergy in the two groups of *H. pylori* positive and negative children.

		H Pylori Test		Chi-Square	P-Value
		No N (%)	Yes N (%)		
Allergy Test	No	142(82.6)	37(97.4)	5.426	0.020
	Yes	30(17.4)	1(2.6)		

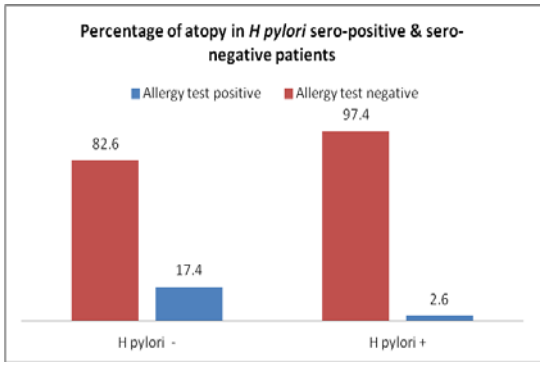

Figure 3. Percentages of aero-allergen sensitivity in the atopic patients

3.3. Triggers of H. pylori-Asthma or Atopy Inverse Association

The effects of sex and age on the negative association between *H. pylori* and atopy or asthma were studied. The obtained results showed that there is a significant negative association between *H. pylori* and doctor diagnosed asthma in the less than nine-year-old age group (*p* value= 0.007); the result for the association of *H. pylori* with atopy was not significant for this age group. Whereas the negative association between *H. pylori* and atopy was significant in males (*p* value= 0.02). (Tables 3, 4). Also, the Odd-Ratio Mantel Haenszel (ORMH) was obtained (ORMH age = 3.5, ORMH gender = 2.2).

Table 3. Frequency of distribution of children according to age and the situation of *H. pylori* and doctor diagnosed asthma.

Age group	Doctor diagnosed asthma	*H Pylori* Test No n (%)	*H Pylori* Test Yes n (%)	Chi-Square	P-Value	Mantel Haenszel Chi-(p-value)	OR	95%CI
≤ 9, y	Negative	87 (79)	12 (52.2)	7.24	0.007	8.94 (0.003)	3.467*	1.357-8.862
	Positive	23 (21)	11 (47.8)					
>9,y	Negative	44 (71)	7 (46.7)	3.18	0.074		1**	-
	Positive	18 (29)	8 (53.3)					

*risk estimate ,**reference value

Table 4. Frequency of distribution of children according to gender and the situation of *H. pylori* and atopy.

Sex	**Allergy Test**	***H Pylori* Test No n (%)**	***H Pylori* Test Yes n (%)**	**Chi-Square+**	**P-Value+**	**Mantel Haenszel Chi-(p-value)**	**OR**	**95%CI**
Male	**Negative**	85(81)	23(100)	-	0.023	4.27 (0.039)	2.169*	0.316-14.896
	Positive	20(19)	0(0)					
Female	**Negative**	57(85.1)	14(93.3)	-	0.679		1**	-
	Positive	10(14.9)	1(0.7)					

+Fisher's Exact Test, *risk estimate, **reference value

3.4. Factors Associated with Asthma

An Asthma and Allergy specialist confirmed all the cases of the disease, and the factors associated with doctor-diagnosed asthma were studied using the multiple logistic regression (MLR) after adjusting for the confounding variables; sex, age, family history, and body mass index (BMI). The obtained results showed that the family history of asthma, *H pylori*, allergy test were significantly shown to have a higher predicting value for asthma (*p* values of 0.001, <0.001 and 0.01, respectively) (Tables 5). Also, the Odd-Ratio Mantel Haenszel (ORMH) of all of the three comparisons were obtained (ORMH *H pylori* test positive = 0.2, ORMH positive family history of asthma = 6.8 and ORMH allergy test positive = 3.1).

Table 5. Factors associated with doctor-diagnosed asthma using the multiple logistic regression (MLR)

Variable	Adj. OR	95% CI (OR)	X^2. Stat. (df)[a]	P. value[a]
Positive H pylori	0.2	0.5-0.1	13.3	0.001
Family history of asthma	6.8	3.4-13.8	30.5	<0.001
Positive Allergy test	3.1	1.3-7.7	6.2	0.01

a Likelihood ratio (LR) test

4. Discussion

A large number of studies demonstrated an inverse relationship between the *H. pylori* infection and asthma or atopy among children, but this relationship remains controversial due to conflicting evidences.

Our study showed that there is an inverse association between *H. pylori* sero-positivity and atopy, and that this association is more apparent in males. Moreover the *H pylori* infection at ages below nine years protect children from being asthmatic. This is in agreement with the previous studies of (Chen and Blaser, 2008; Shiotani *et al*., 2008). Furthermore the results of the multiple logistic regression showed that the main risk factors for the doctor-diagnosed asthma were *H. pylori* sero-negativity, a positive atopy test, and a positive family history of asthma. These results were consistent with other studies.

David Strachan, the founder of the historic hygiene hypothesis, was the first to postulate that the infections acquired in early childhood could prevent atopy. In the study published in 1989, he noticed that hay fever was inversely related to the number of children in the household, and to the number of older children in families. He speculated that the declining of family size associated with the improvement of cleanliness had reduced the prevalence of cross infections in families, leading to a prevalence of the atopic disease, as is the case for hay fever (Strachan, 1989). Some years later, von Mutius *et al.* (Von Mutius *et al.*, 1994) elaborated that hypothesis studying the prevalence of atopic sensitization (screened by skin prick tests) in two groups of people: children living in West Germany, and children living in East Germany, in relation to the number of siblings. He observed that atopic sensitization was three times more prevalent in West Germany than in East Germany, because of the higher standard of living.

Several case control and cross-sectional studies have investigated the relationship between the *H. pylori* infection and allergic diseases. Most of them have been conducted in industrialized countries (United States (Reibman et al., 2008), Finland (Kosunen *et al*., 2002), Great Britain (Jarvis *et al.*, 2004), Japan (Imamura *et al*.,

2010)), where atopy was found to be more common, and the decreasing trend of the *H. pylori* infection being effective. Based on these studies and others as well, the current study concludes that there is some evidence of an inverse association between atopy/allergic diseases and the *H. pylori* infection (Chen and Blaser, 2008), even though further investigations are still needed.

Furthermore, several studies demonstrated and confirmed the development of an allergic disease, or increase of IgE after the *H. pylori* eradication. A Korean study demonstrated increased levels of IgE related, non IgE related allergies as well as a subclinical rise of IgE levels in patients after the *H. pylori* eradication, compared with the *H. pylori*-positive patients without eradication and *H. pylori* negative controls (Lee *et al*., 2015).

In addition, some studies stated that the inverse relation between *H. pylori* and allergy is conditional; for instance one study proved that the significant negative association was demonstrated in men, but not in women, underlining a difference in the negative association in relation to sex, which suggests a different immune response to *H. pylori* in women more than in men (Shiotani *et al*., 2008). Other studies showed that the negative association was stronger in child asthma than in adult asthma, as the adult asthma may be multifactorial and child asthma is mostly attributed to atopy (Chen and Blaser, 2008); for instance, Blaser considered that the inverse association observed with early life asthma (not with long-standing asthma seen in adults) supported the role of *H. pylori*, since the effect of *H. pylori* might be less important in adult-onset asthma due to the much more heterogeneous nature of adult asthma. However, the confounding factors that could influence the association are not fully ruled out.

Potential mechanisms by which *H. pylori* could alter the presentation of asthma include immune modifications, or an effect on the gastroesophageal reflux disease (GERD). The exogenous infection and microbial substances including the *H. pylori* infection may elicit a Th1-mediated immune response, which suppresses Th2 responses (Amedei *et al*., 2006; Codolo *et al*., 2008). The lack of adequate stimulation of the Th1 might result in an overactive Th2, which in turn leads to allergy (D'Elios and Bernard, 2010). Moreover, the acquisition of *H. pylori* may be of importance in the induction of regulatory T cells, which could effectively reduce the possibility of allergy (Moyat and Velin, 2014). The immune-modulatory properties that allow the bacteria to persist for decades in infected individuals in the face of a vigorous, yet ultimately non-protective, innate, and adaptive immune response may at the same time confer protection against allergies, asthma, and inflammatory bowel diseases. Regulatory T-cells mediating peripheral immune tolerance have emerged as key cellular players in facilitating persistent infection as well as protection from allergies in humans as appears from the observational studies and in mice as the experimental studies have shown (Arnold and Anne Müller, 2012; Slomiany and Slomiany, 2014). Associations between GERD and asthma are also well-established (Harding, 2005). Longitudinal studies show that asthma is a risk factor for the development of GERD, and that GERD can trigger asthma. *H. pylori*; in particular CagA+ strains, are inversely associated with GERD (Haruma *et al*., 2000; Peek Jr and Blaser, 2002; Blaser and Atherton, 2004). Although we did not specifically investigate GERD in this study, there is a possibility that the inverse association between *H. pylori* and asthma reflects protection from GERD.

H. pylori may be asymptomatic; however, children infected with *H. pylori* can manifest gastrointestinal diseases. Although this study showed a significant association between *H. pylori* related gastrointestinal symptoms (except for poor growth; data not shown) and *H. pylori* seropositivity, controversy persists regarding testing (and treating) for the *H. pylori* infection in children with recurrent dyspepsia, chronic idiopathic thrombocytopenia, regurgitation, gastric ulcer and/or anemia. Because of the not conclusive evidences of the role of *H. pylori* in childhood gastrointestinal disease, several studies have demonstrated that *H. pylori* infection is not associated with specific symptomatology in children (Pacifico *et al*., 2010). Therefore, the identification of children with *H. pylori*-associated gastritis on the basis of clinical presentation alone is not possible. Based on the best available evidence, testing for (and treating) *H. pylori* infections should be performed in children with endoscopically proven duodenal ulcer. Evidence from studies on adults supports the recommendation that testing for *H. pylori* should also be performed in children with a documented gastric ulcer. Endoscopy and biopsy are also recommended for children with persistent symptoms (Patel *et al*., 1994)

This study has several limitations that should be mentioned, for instance the cross-sectional study design. Because this study only checked the history of asthma, it could not exclude the possibility of the development of asthma before the *H. pylori* infection. However, as *H. pylori* acquisition is known to occur early in life, the development of asthma was presumed to be the later event in the present study. Most of the subjects were not highly-educated living in a rural area. This might have caused a selection bias. Recall bias may have occurred, as our data on the history of allergic diseases (hay fever, eczema and food allergy) are depended on self-reports. Furthermore, the assessment of patients for the *H. pylori* infection using the biopsy method, which has a higher sensitivity and specificity than serology tests, is needed to prove the results. However, after adjusting for those factors, the *H. pylori* infection was inversely related to asthma and atopy. Performing more studies with larger sample sizes is necessary to confirm these results.

5. Conclusion

In conclusion, our data suggest that *H. pylori* is inversely associated with atopy and asthma. It also shows that this inverse association was more apparent in males and in the less-than-nine age group. Moreover, the *H. pylori* seronegativity, positive family history of asthma, and positive atopy test are all triggering factors for asthma (after removing the confounding factors). Obviously, a precise recognition of the correlation between the *H. pylori* infection and asthma in young children could play an important role in the recognition of the physiopathology of not only asthma, but of other allergic diseases as well. This would offer potentially helpful new treatments for allergic diseases. Therefore, we recommend a careful consideration

of whether to eradicate *H. pylori* in young patients with asthma risk factors, as *H. pylori* may play a role in reducing the risk for asthma. Further prospective studies are warranted to clarify the underlying mechanisms.

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