

Helicobacter pylori seropositivity in children with atopic dermatitis as sole manifestation of food allergy

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A positive association between *Helicobacter pylori* antibodies and food allergy presenting with gastrointestinal symptoms has recently been reported. A subset of a *H. pylori* strain possesses an antigen, CagA, as a virulence factor. Anti-*H. pylori* and anti-CagA IgG titre have been determined in children with atopic dermatitis (AD) as the sole clinical manifestation of food allergy. In this study, thirty patients with AD as the sole clinical manifestation of food allergy were examined (group A). For comparative purposes, 30 patients affected by food allergy with gastrointestinal symptoms (group B) and 30 affected by atopic asthma (group C) were studied. Anti-*H. pylori* and anti-CagA immunoglobulin G (IgG) were determined in all individuals by means of the enzyme-linked immunosorbent assay. The anti-*H. pylori* IgG titre was significantly higher in group A and group B vs. group C ($p < 0.05$); no significant difference was detected between group A and group B ($p > 0.05$). No significant difference in anti-CagA titre was found between the groups. These data demonstrate a positive association between *H. pylori* antibodies and AD as the sole manifestation of food allergy. Further investigations are needed to evaluate the cause–effect relationship between *H. pylori* seropositivity and AD.

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In children, *Helicobacter pylori* infection has been shown to play a role in the pathogenesis of gastritis and duodenal ulcer; moreover, it has been recognized as a cofactor in the development of protein-losing enteropathy, chronic diarrhoea, short stature and gastric lymphoproliferative disease (1–3). Microbiological and histological methods, as well as polymerase chain reaction and urea breath test, have been used to assess *H. pylori* infection (4); however, it has been stressed that, in children, the specific immunoglobulin G (IgG) test may provide a useful tool in the diagnostic work-up of *H. pylori* infection (3, 5–7). All *H. pylori* strains express several virulence factors, such as a potent urease, flagella, and various adhesins, which are thought to be important for survival in the gastric environment and tissue-specific colonization. A subset of

strains also possesses a surface-exposed immunodominant antigen known as CagA (cytotoxin-associated gene A) (8).

Atopic dermatitis (AD) is a complex multifactorial disease, characterized by a chronic pruritic cutaneous inflammation. This condition occurs predominantly in infancy and childhood, with an increase in frequency over the last few years (9). Diagnostic criteria for AD were set up by Hanifin and Rajka in 1980 (10). The pathogenetic mechanisms of AD remain to be elucidated, however, they include food allergy reaction, infections, and environmental, psychoneurologic and metabolic factors (11–14).

We recently demonstrated a positive association between *H. pylori* antibodies and food allergy in children with gastrointestinal symptoms (3). The aim of the present study was to

investigate anti-*H. pylori* IgG in children with AD as the sole clinical manifestation of food allergy.

Methods

Patients

This prospective study was carried out in our Pediatric Gastroenterology Unit between January 1997 and January 1999. The study population initially comprised 35 consecutive patients (group A: 25 male, 10 female, age range 4–11 yr, mean age 6.9 yr) affected by AD as defined according to the criteria of Hanifin and Rajka (10). No signs or symptoms of gastrointestinal disease or asthma had been reported. These patients were referred to us by pediatricians in order to investigate food allergy. All patients were positive to skin-prick tests with food allergens (casein, lactalbumin, beta-lactoglobulin, ovalbumin, rice, soya, wheat and fish) diluted in glycerine at a 1/100 concentration (Lofarma, Milan, Italy). The immunoglobulin E (IgE) paper radioimmunosorbent test (PRIST) method was used to determine total IgE levels, which in 34/35 (97%) of the patients were higher than the mean $\pm$ SD. The Phadebas radioallergosorbent test (RAST) method (RIA, Pharmacia Diagnostics, Uppsala, Sweden) was used to determine specific IgE levels, which were positive in 29/35 (83%) of patients with positive skin-prick tests. Both the specific IgE and prick tests were more frequently positive for casein, lactalbumin, beta-lactoglobulin, egg white, rice and soya. All 35 patients were put on a diet excluding the food for which the skin-prick test had shown a positive result. After three weeks on the diet a double-blind provocation test was carried out with suspected foods on 32/35 (91%) children who had shown a clinical improvement, in order to confirm the diagnosis of food intolerance. The food challenge was carried out in the fasting state, starting with a dose unlikely to provoke symptoms (125 mg to 500 mg of lyophilized food). The dose was doubled every 15–60 min, depending on the type of reaction suspected. Patients were randomly assigned either to opaque white capsules with lyophilized food or to the placebo (sucrose), with a 'washout' period of one week. After oral challenge, patients were closely monitored for 4–6 h. However, if the blinded challenge was negative, it was further confirmed by open feeding, under close observation, in order to rule out false-negative results. To better control a variety of potential confounding factors, an equal number of food antigen and

placebo challenges were employed. A standardized scoring system was used to reveal cutaneous symptoms (15). Of 32 children, 30 (86%), who had shown clinical improvement, were positive to the double-blind controlled placebo food challenge (DBCPFC). Only these 30 patients were enrolled in the study, considering DBCPFC to be the 'gold standard' for the diagnosis of food allergy.

Two groups were studied for comparative purposes. The first, group B, comprised 30 consecutive children (18 male, 12 female, age range 5–12 yr, mean age 7.2 yr) affected by food allergy, and presenting with gastrointestinal symptoms, namely, failure to thrive, vomiting or chronic diarrhoea. No signs or symptoms of cutaneous disease or of respiratory tract infections were referred. The diagnosis of food allergy was made with skin-prick tests, PRIST and RAST and was confirmed by DBCPFC, as in the patients with AD. The second group, group C, comprised 30 consecutive patients (16 male, 14 female, age range 6–12 yr, mean age 7.4 yr), with moderate to severe bronchial asthma, based on episodic cough and wheeze with response to β_2 -agonist persisting for at least 6 months with no history of any other disease (16). No signs or symptoms of cutaneous and/or gastrointestinal diseases were referred. The patients were considered to be atopic following at least one positive skin-prick test or one positive RAST analysis to a panel of common inhalatory allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Graminaceae mix*, *Alternaria tenuis*, *Parietaria officinalis*, *Parietaria Giudaica*, cat and dog dander) (17). Total IgE levels were higher than the mean $\pm$ SD in 27/30 (90%) patients. All patients were negative on skin testing to food antigens.

There were no differences in social and ethnic backgrounds between the children enrolled in the study.

Serology

Serum samples, in all patients, were stored at -20°C until analysis.

Immunoglobulin G antibodies to *H. pylori* were determined by means of commercial sandwich-type enzyme immunoassay (Helori-test IgG Chromo, Eurospital, Trieste, Italy), according to the manufacturer's instructions. The positive cut-off was 21.01%, established on the basis of the mean plus 3 SD of the control group, obtained from 30 children with recurrent abdominal pain (RAP), as defined by the criteria of Apley and Naish (18). All patients had negative prick tests to

food antigens and a negative response to a diet without cow's milk protein or egg (19). To establish the presence of *H. pylori* infection, patients with RAP underwent oesophagogastrroduodenoscopy during which two antral biopsies were collected (20). Culture, urease test and histological identification of *H. pylori* were negative. The negative cut-off was 16.41%, based on the mean of the control group plus 2 SD.

To determine IgG antibodies against CagA in serum samples, a commercial enzyme immunoassay was used (Helori CTX IgG), according to the manufacturer's instructions. Also for this test, we used the control group to establish the range of normal values: the positive cut-off was 9.34 units (mean of control group plus 3 SD); the negative cut-off was 7.07 units (mean of control group plus 2 SD).

Statistics

Differences between groups with respect to age and sex were analyzed by one-way ANOVA. Comparison of anti-*H. pylori* IgG and anti-CagA IgG serum levels between groups was performed using the Mann-Whitney *U*-test and the Kruskal-Wallis test. Values are expressed as mean $\pm$ SD. Values with a *p*-value of < 0.05 were considered significant.

Ethical considerations

The parents of all patients gave informed written consent to the study, which was approved by the Ethics Committee of the Institute of Pediatrics of the University 'La Sapienza' of Rome, Italy.

Results

No statistically significant differences were found between groups with respect to age and sex.

In group A, 17/30 (56%) had values above the cut-off point of 21.43%, thus indicating the presence of anti-*H. pylori* IgG; 2/30 (7%) had borderline values; and 11/30 (37%) had negative results. The mean anti-*H. pylori* IgG titre was $28.42\% \pm 23.91$ SD. In group B, 11/30 patients (37%) had positive values; 10/30 (33%) were borderline; and 9/30 (30%) were negative. The mean anti-*H. pylori* IgG titre was 25.69 ± 21.12 SD. In group C, 3/30 patients (10%) had positive values; 2/30 (7%) were borderline; and 25/30 (83%) were negative. The mean anti-*H. pylori* IgG titre was 10.65 ± 11.98 SD. The anti-*H. pylori* IgG titre was significantly higher in group A and group B vs. group C ($p < 0.05$); no significant

difference was detected between group A and group B ($p > 0.05$) (Fig. 1).

As far as concerns CagA antibodies in group A, 2/30 patients (7%) had values above the cut-off of 9.04, thus indicating the presence of anti-CagA IgG; 3/30 (10%) were borderline; and 25/30 (83%) negative. The mean anti-CagA IgG titre was 3.1 ± 2.19 SD. In group B, 3/30 (10%) had positive values; 3/30 (10%) were borderline; and 24/30 (80%) negative. The mean anti-CagA IgG titre was 4.93 ± 4.22 SD. In group C, 2/30 (7%) had positive values; 2/30 (7%) were borderline; and 26/30 (86%) negative. The mean anti-CagA IgG titre was 3.4 ± 2.70 SD. The anti-CagA titre did not differ significantly between the three groups.

Discussion

Our results show an increase in anti-*H. pylori* IgG serum levels in children with AD presenting as the sole manifestation of food allergy. Data in the literature on the relationship between infections and allergic diseases remain to be clarified because it has been reported that infections may prevent or induce atopy (21). With respect to *H. pylori*, some data seem to suggest that the increase in *H. pylori* antibodies may occur prior to AD. *H. pylori*, in damaging gastric mucosa,

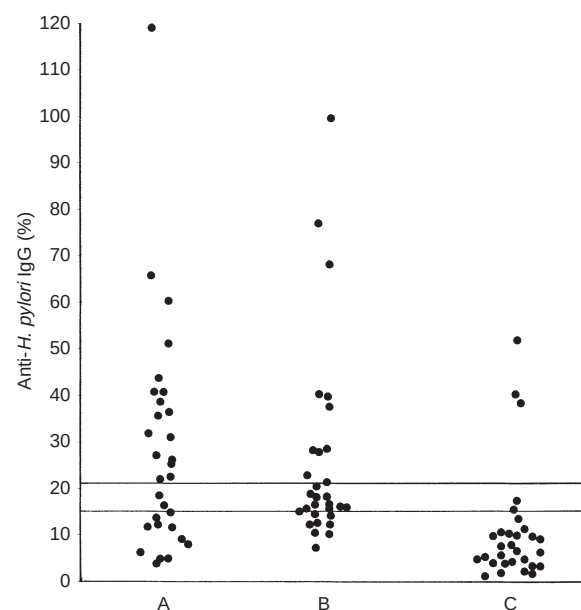


Fig. 1. Scattergram showing results of anti-*Helicobacter pylori* immunoglobulin G (IgG) assay in patients with atopic dermatitis as the sole manifestation of food allergy (group A); food allergy with gastrointestinal symptoms (group B); and atopic asthma without food allergy (group C). The two lines indicate mean +3 SD of *H. pylori*-negative controls at 21.01%, and the mean +2 SD at 16.41%. Group A vs. group B: $p > 0.05$; group A and group B vs. group C: $p < 0.05$.

may trigger a food allergy reaction (22). Later, an increase in IgE serum levels induces synthesis and release of cytokines, sustaining chronic allergic inflammation, such as AD (23). Moreover, it has been stressed that *Staphylococcus aureus* may promote AD by an IgE-mediated immune response (11). It is tempting to hypothesize that *H. pylori* may also induce a similar immunological event. Finally, Murakami et al. have reported on a case of AD and *H. pylori* infection in a 14-year-old girl: AD was successfully treated by eradication of *H. pylori* without any treatment for the skin lesions (24). However, other data seem to suggest that food allergy may occur before the increase in anti-*H. pylori* IgG serum levels. In patients with food allergy, the gastric mucosa may be hyperaemic and edematous and it is well-known that such gastric mucosa lesions may promote *H. pylori* adhesion (25).

Data emerging from the present investigation indicate that anti-*H. pylori* IgG serum levels do not differ significantly between patients with AD and patients with gastrointestinal symptoms. These data are in agreement with other findings reported in the literature indicating that an increase in anti-*H. pylori* IgG serum levels may be present even in the absence of typical gastrointestinal symptoms or diseases (i.e. myocardial infarction, hepatitis A and B, and severe neurologic impairment) (7, 26–28). Moreover, our results show significantly higher serum levels of anti-*H. pylori* IgG in children with food allergy (presenting with AD or with gastrointestinal symptoms) than in patients with atopic asthma. Thus, it is tempting to suggest that the increase in *H. pylori* antibodies is not related to atopic condition but to food allergy. This would not be surprising because *H. pylori* and food allergy both have the stomach as a common target-tissue.

In the present study we were unable to detect a significantly higher titre of CagA antibodies in the patients with AD. These results are in agreement with our previous findings in patients presenting with food allergy with gastrointestinal symptoms (3). Conversely, in adults, Figura et al. (29) have shown that CagA seropositivity is significantly higher in patients with food allergy than in controls. It might be hypothesized that such data depend on the difference in gastric factors between children and adults with food allergy.

In conclusion, our data show a significant association between *H. pylori* seropositivity and AD as sole manifestation of food allergy. A follow-up evaluation after treatment is now underway to clarify the cause-effect relationship between *H. pylori* seropositivity and AD.

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