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Association between infection with *H. pylori* and atopy in young Ethiopian Children: a longitudinal study

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Abstract

Background: Epidemiological evidence from developed countries indicates that *Helicobacter pylori* infection correlates with a reduced risk of atopy and allergic disorders, however limited data are available from low-income countries.

Objective: We examined associations between *H. pylori* infection in early childhood and atopy and reported allergic disorders at the age of 6.5 years in an Ethiopian birth cohort.

Methods: A total of 856 children (85.1% of the 1006 original singletons in a population-based birth cohort) were followed up at age six and half years. An interviewer-led questionnaire administered to mothers provided information on demographic and lifestyle variables. Questions on allergic disease symptoms were based on the International Study of Asthma and Allergies in Children (ISAAC) core allergy and environmental questionnaire. Serum samples were analysed for total IgE levels and anti-*H. pylori* cytotoxin associated gene A (CagA) IgG antibody using commercially available ELISA kits. Stool samples were analysed for *H. pylori* antigen using a rapid immunochromatographic test. The independent effects of *H. pylori* infection (measured at age 3, 5 and 6.5 years) on prevalence and incidence of atopy and reported allergic disorders (measured at age 6.5 years) were determined using multiple logistic regression.

Results: In cross-sectional analysis, current *H. pylori* infection at age 6.5 years was inversely, though not significantly, related to prevalence of atopy and 'any allergic condition' at age 6.5 years. However detection of *H. pylori* infection at any point up to age 6.5 years was associated with a significantly reduced odds of both atopy and 'any allergic condition' (adjusted OR AOR, 95% CI, 0.54; 0.32 to 0.92, p=0.02, and 0.31; 0.10 to 0.94, p=0.04, respectively). In longitudinal analyses, *H. pylori* infection at age 3 was inversely associated with incidence of atopy (AOR, 95% CI, 0.49; 0.27 to 0.89, p=0.02). Furthermore, among *H. pylori* infected children, those with a CagA+ strain had a more pronounced reduction in odds of atopy (AOR=0.35 vs. 0.63 for CagA+ vs. CagA-) and this reduction reached borderline significance.

Conclusion: These data are consistent with the hypothesis that early exposure to *H. pylori* is inversely associated with atopy and allergic conditions. A possible modest protective association against atopy was observed in those infected with a more virulent CagA+ strain of *H. pylori*.

Key words: *Helicobacter pylori*, atopy, allergic disorders, birth cohort, Ethiopia

Introduction

The prevalence of IgE-mediated atopy and allergic diseases has been increasing considerably over the past 30 years, and this is particularly apparent in developed countries(1, 2). In conjunction with evidence from other developing and transitional societies (3, 4), this indicates that factors associated with urbanization and/or relative affluence are likely to be responsible for these emerging allergic disorders. A potential explanation for this was given by Strachan, who first proposed the hygiene hypothesis (5). The 'hygiene hypothesis' (now revised as the 'old friends hypothesis') (6) postulates that the rise of allergic disease in recent decades is attributable to a decrease in exposure to certain microbes and infections during childhood, and has gained wide acceptance (7). Microbial infections, which are less prevalent in populations with improved lifestyles and better hygiene, may play a protective role in the aetiology of asthma and allergy (8, 9). Several viral and parasitic pathogens, including influenza viruses and helminths have been reported to play a role in protection against asthma and allergy (10, 11). *Helicobacter pylori* is a common bacterium, which is estimated to be present in the stomach of around half the world's population. It usually colonises the gastric mucosa from early childhood, where it persists lifelong unless treated with antibiotics. It is the major cause of peptic ulceration and gastric cancer, however disease occurs in only 10-15% of cases and the infection usually remains asymptomatic. *H. pylori* has co-evolved with humans over thousands of years, however the prevalence of the infection (particularly amongst children in developed countries) has been declining rapidly over the last two decades (12-14). Important epidemiologic evidence for the role of *H. pylori* in the hygiene hypothesis has recently emerged, and the infection has been associated with a reduced risk of allergic disorders including asthma (15), wheeze (16), eczema (17-19) , and atopy (20-22). This association has also been reported to be stronger with more pathogenic strains of *H. pylori* which express the virulence factor cytotoxin

associated gene A (CagA) (23, 24). Infection with these strains reportedly induces a higher-level T-helper 1 (Th1) cellular immune response (24, 25), which may counterbalance the pro-allergic Th2 response. CagA+ strains usually also co-express the most active form of the vacuolating cytotoxin A (VacA), and this is important in the induction of immunosuppressive regulatory T cell (Treg) responses (26). Higher frequencies of IL-10-secreting Tregs were shown to be present in the peripheral blood of *H. pylori*-infected patients in the UK, and serum IgE concentrations were lowest when there was a strong Treg response. Mechanistic *in vitro* experiments showed that the systemic IL-10+ Treg response is likely to play an important role in *H. pylori*-mediated protection against allergy in humans (26). A number of studies with mouse models of allergy and asthma have very convincingly demonstrated that *H. pylori* infection is protective and that VacA-induced Tregs play a major role (27-29). Despite this convincing evidence, however, others have reported no epidemiological association between the infection and asthma (30, 31) or atopy (31, 32). This inconsistency may be attributed to the way in which allergic disease is defined or the heterogeneity of the study designs and study populations.

Our recent meta-analysis, using 16 observational studies showed a significant protective effect of *H. pylori* infection against objectively measured atopy (33). However, most studies included in this analysis were cross-sectional, with only two prospective studies (34, 35), based in adult high-income country populations, and lacked data on children from low-income countries. This is important as infection with *H. pylori* is usually acquired very early in childhood (36), when the immune system is developing. It is possible that timing of exposure to *H. pylori* infection may be important in modulating its effects, and any long-term protection that arises from effects occurs during the crucial early period of immune development.

Our previous work on the Butajira Birth Cohort in rural Ethiopia demonstrated an inverse association of *H. pylori* infection with skin sensitization to either *D. pteronyssinus* or cockroach allergen (37) measured by skin prick test at age 5. Although this method provides a convenient test for atopy in epidemiological studies (38), and the allergens used are believed to be predominant in Ethiopian populations(8), this approach will not necessarily identify all cases of atopy. It is therefore important to consider atopy as defined in terms of total serum IgE, since it has been suggested that this provides an overall estimate of allergic disorders (39, 40). We therefore expanded our previous observations by collecting additional data on total serum IgE levels when the child reached 6.5 years, in order to investigate a possible association between atopy (defined by a raised total serum IgE level) and *H. pylori* infection. Additionally, and for the first time in this cohort, we also tested whether the presence of a CagA+ *H. pylori* strain was associated with atopy and allergic conditions.

METHODS

Study Setting and Design

A detailed description of the original Butajira birth cohort study has been published previously (17, 41). Briefly, the birth cohort is nested in the Butajira Demographic Surveillance Site (42) which covers a sample of nine rural and one urban administrative units in and around the town of Butajira in Southern Ethiopia (43). Between July 2005 and February 2006, all women in the DSS aged 15–49 and in their third trimester of pregnancy were identified by the DSS fieldworkers and invited to participate in the study. Of the 1,234 eligible women, 1,065 were recruited (86% of those eligible) and all live singleton babies born to these women (n = 1006) were followed-up as a birth cohort (figure 1).

Measurement and Data collection

At age 6.5 follow-ups visits took place, where the same project data collectors visited the child at home and collected information related to allergic outcomes. Questions on allergic disease symptoms were based on the International Study of Asthma and Allergies in Children (ISAAC) core allergy and environmental questionnaire (44), and included wheeze (*'In the last 12 months has your child had wheezing or whistling in their chest?*'), asthma (*'In the last 12 months has your child had asthma*?'), hay fever *('In the last 12 months has your child had problems with sneezing or running nose (when not affected by cold or flu), or problems with itchy watery eyes?')* and eczema (*'In the last 12 months has your child ever had an itchy skin rash which has affected the skin creases, eg, front of the elbow, behind the knees, the front of the ankles, around the neck, or around the eyes?')*. The questionnaire also asked about various potential confounders including environmental factors (sanitation and water supply, household roof/wall/floor type, indoor smoking, presence of animals, insecticide use, cooking facilities), asthma and allergy in the family, household size, child's sleeping place, siblings, birth order, and child's use of medication (use of paracetamol and antibiotics).

In addition to the questionnaire data, at the age 3, 5 and 6.5 year visits, mothers were also asked to collect a faecal sample from their child using a leak-proof plastic container. The samples were then transported for analysis in the Butajira Health Center laboratory to ascertain the child's *H. pylori* and intestinal parasite infection status. Furthermore, at the 6.5 year visit, a 5 ml blood sample was collected from each child using a vacutainer tube. Serum samples were separated within 2 hrs. of collection at Butajira hospital and then transported to the Ethiopian Public Health Institute (EPHI) Research Laboratory, Addis Ababa for serum CagA and IgE ELISA analyses.

Laboratory analyses

***H. pylori* status**

H. pylori status was evaluated using the commercially available SD Bioline *H. pylori* stool antigen test (Standard Diagnostics, Inc) according to the manufacturer's instructions. A portion of faeces (approximately 50mg) from each stool sample was mixed with assay diluent solution, until the sample had been dissolved, before allowing the suspension to settle for 5 minutes at room temperature. 100 µL of the liquid phase of the sample was placed on the *H. pylori Ag* test strip. The test results were checked after 15 minutes, where one red line indicated a negative result and a double red line indicated a *H. pylori* positive result.

Additionally, all faecal samples were examined qualitatively using the modified formol-ether concentration method to ascertain the child's intestinal parasites infection status.

***H. pylori* CagA status**

Serum anti-CagA IgG antibody status was assessed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Genesis Diagnostics Ltd, UK), as per the manufacturer's instructions. The optical densities of the wells were measured using a microplate reader at 450nm. Values above the 6.25 U/ml standard were defined as positive. According to the manufacturer, the sensitivity and specificity of this kit are 96.3% and 96.7%, respectively.

Total IgE analysis

Total IgE levels were measured using a commercially available ELISA kit (Omega diagnostic, UK), according to the manufacturer's instructions. The optical densities (ODs) of the standards, positive control and samples were measured using a microplate reader at 450nm. Elevated IgE levels were determined based on the manufacturer's recommended threshold by age range. For this study total IgE levels greater than 144 IU/ml for age 5-10 were considered as the cut-off value to define atopy.

Outcome definition

Atopy was defined in this study as an elevated total serum IgE. A total serum IgE concentration of ≤ 144 IU/ml for children aged 5-10 years was considered to be normal based on reference ranges provided by the manufacturer's recommended threshold by age range.

'Any allergic condition' was defined as a positive response to one or more of the questions on asthma, hay fever and eczema in the past 12 months, at years 6.5 follow-up.

Statistical analysis

Data were double entered into EpiData 3.1 (EpiData, Denmark). The datasets were cleaned, coded and merged ready for analysis using Stata 12 (Statacorp, College Station, Texas, USA).

Prior to investigating the association between *H. pylori* infection and atopy, univariate analyses were used to identify the possible confounders. Variables that were associated with both exposure and outcome variables in the crude analysis using statistical significance at p value <0.2 were considered to be possible confounders. Additionally, we included variables previously shown to be associated with atopy and /or elevated total IgE in the literature; child's sex (45), smoking (46), antibiotic use (47) and intestinal parasite status (48)

We estimated odds ratios (ORs) associated with *H. pylori* positivity for atopy and 'any allergic condition' using multivariate logistic regression. The ORs were adjusted for *a priori* and potential confounders listed in Table S1. The same approach was used in a separate set of analyses to assess the effect of CagA *H. pylori* strain infection on atopy and self-reported allergic symptoms for all available children at year 6.5, by creating a new exposure variable with categories representing different combinations of *H. pylori* and Cag A exposure status: 'Negative' (negative for both *H. pylori* and CagA strain), '*H. pylori positive and CagA Negative* ' (positive for *H. pylori* but negative for Cag A strain) and '*H. pylori positive and CagA positive'* **(**positive for both *H. pylori* and CagA strain). At age 6.5 year follow up visit, the prevalence of self-reported allergy symptoms was low, ranging from 0.5% for eczema and hay fever to 2.2% for wheeze, we therefore create a new outcome variable 'Any allergic condition at age 6.5' (i.e. positive response either to wheeze, or eczema, or hay fever in the past 12 months, at years 6.5 follow-up).

For longitudinal analysis of incident atopy between ages 3 and 6.5, those children without atopy (defined as 'negative to any skin sensitization test (SPT), i.e. skin prick test to either *Dermatophagoides pteronyssinus* or cockroach allergen <3mm diameter) at age 3 were selected for analysis and incident atopy defined as a raised total serum IgE level at the year 6.5 follow up. Furthermore, children with self-reported allergic symptoms such as wheeze; eczema and hay fever at ages 3 (positive response to wheeze, eczema, and hay fever in the past 12 months, at years 3, and ever at year 3) were excluded for analysis of incident atopy at the year 6.5 follow up.

Covariates were kept in the model if they changed the coefficient of exposure (*H. pylori* infection) by > 10% or if they were independently associated with the outcome at $p < 0.10$. Probability values < 0.05 were considered statistically significant for main effects

Sensitivity analysis was done to compare the distribution of demographic and life style variables between study subject who have complete outcome data (i.e. “complete-case”) and the "all respondents" populations.

Ethical Approval

The study was approved by the Institutional Review Board (IRB) of Addis Ababa University, College of Health Sciences, Ethiopia, and the University Of Nottingham Medical School Ethics Committee. Written, informed consent was obtained from the mothers after they have been clearly informed about the study, and in keeping with the requirements of the College of Health Sciences IRB all women and their children were reimbursed for health care costs. Children were also requested to give assent and were informed of their right to refuse to participate in the study and to withdraw at any time during the study without jeopardizing their right of access to other health services. Invasive procedures such as collection of blood samples were fully explained to parents and children, and were carried out using sterile disposable materials

Results

Description of cohort participants followed-up at age 6.5 years

A detailed description of the cohort at years 1, 3, and 5 is reported elsewhere (17, 37, 43). At 6.5 years, a total of 856 singleton children were successfully followed-up (85.1% of the original cohort at birth, and 99.3% of those available at year 5 follow-up), of whom 713 had total serum IgE measurement and 848 had *H. pylori* data at the 6.5 year follow up visit (Fig 1).

Demographic and lifestyle characteristics of study participants at 6.5-year follow-up visit

Of children enrolled at the 6.5 year follow up visit, 51.2% (434/848) were male and the majority, 88.2% (748/848) were from a rural area. Maternal demographic characteristics showed that 47.4% (402/848) of the mothers belonged to the Meskan ethnic group, 78.4% (665 /848) were Muslim, 71.6% (607/848) were illiterate and 83.8% were housewives (709/848). Most mothers (61.3%, 520/848) reported using piped water as their primary drinking source. Selected early life characteristics at 2 months and 1 year of age showed that 57.5% had been vaccinated at 2 months and only 11.7 (99/848) had received vitamin A supplementation at 1 year (online supplementary: Table S1).

Comparing the distribution of demographic and life style variables between all study subjects and those who have complete outcome data (i.e. "all respondents" and “complete-case”), found similar distribution patterns in relation to demographic and life style characteristics (online supplementary: Table S2).

Prevalence of atopy and allergic conditions among children followed up at age 6.5

The prevalence of atopy among children enrolled at the 6.5-year follow up visit was 18.1% (129/713), with a higher proportion among rural (20.0%) than urban (6.1%) residents, $p<0.05$ (Table 2). Using an ISAAC core allergy and environmental questionnaire, wheezing was reported in 2.2% (18/806) of children whilst eczema and hay fever was reported in less than 1% of the children (Table 1). When any combination allergic condition considered, the prevalence reached 3.0% (24/806), with a similar proportion among urban and rural children, (3.1% vs. 3.0% in urban and rural, respectively, $p= 0.93$, Table 1).

Potential confounders of atopy

The main outcome variable showed no statistically significant association with most demographic and lifestyle variables, including child's sex, history of vaccination, use of antibiotics, and intestinal parasite infection at age 6.5. Neither were family history of allergic conditions (maternal and paternal), common environmental factors such as household roof type, indoor smoking, insecticide use, and child's sleeping place significantly associated with atopy. However, atopy was significantly associated with place of residence, indoor cooking, presence of large animal(s) inside the house, and higher numbers of older siblings (Table S3).

Association between *H. pylori* infection and atopy or allergic conditions at age 6.5

The prevalence of atopy was lower in children currently infected with *H. pylori* (15.2%) than in those not infected (18.6%), but this did not achieve statistical significance (p = 0.46). In multivariate analysis adjusted for *a priori* confounders, *H. pylori* infection at age 6.5 was inversely, though not significantly, related to prevalence of atopy and 'any allergic condition' at age 6.5 years (adjusted OR, 95% CI, 0.87; 0.45 to 1.70, p=0.69). When the analysis was restricted to those children who had *H. pylori* infection at age 3, 5 and 6.5 years, or those who were ever infected up to age 6.5, a more pronounced reduction in odds of atopy and 'any allergic condition' was demonstrated. This reduction reached statistical significance (adjusted OR, 95% CI, 0.54; 0.32 to 0.92, p=0.02, and 0.31; 0.10 to 0.94, p=0.04, respectively Tables 2&3).

Association between CagA+ *H. pylori* infection with atopy and allergic condition at age 6.5

Separate logistic regression models related atopy (outcome) to the individual estimates of CagA *H. pylori* infection status at age 6.5 years (exposure). These showed a non-significant reduction in the odds of atopy among *H. pylori*+ CagA- children (Adjusted OR=0.63, 95%CI, 0.21 to 1.87, p=0.63) compared to non-infected children. Children infected with CagA+ strains of *H. pylori* showed a more pronounced reduction in odds of atopy and reached a borderline significant association (Adjusted OR=0.35, 95%CI, 0.12 to 1.01, p=0.051) (Table 4).

A broadly similar pattern, but non-significant inverse association was also observed between exposure to CagA+ *H. pylori* and any combination of self-reported allergic symptoms ('any allergic condition') at age 6.5 (Adjusted OR=0.73, 95%CI, 0.10 to 5.56, p=0.76) (Table 5).

Effects of *H. pylori* infection on incidence of atopy between ages 3 and 6.5: Longitudinal Analysis

Table 6 presents the models for the longitudinal analysis between *H. pylori* infection and atopy Data are presented for the population without atopy (defined as 'negative to any skin sensitisation test (SPT)) at age 3 to examine the effect of *H. pylori* infection at age 3 on the new onset of atopy (defined as raised total serum IgE level) at age 6.5. *H. pylori* infection at age 3 was inversely associated with incident atopy, after control for *a priori* and identified confounders (adjusted OR, 95% CI, 0.49; 0.27 to 0.89, p=0.02) (Table 6). Adjustment for other potential confounders did not materially change the odds ratio.

Discussion

This study provides further evidence from a low-income, population-based birth cohort that current *H. pylori* infection at age 6.5 is inversely, though not significantly, related to prevalence of atopy (defined as a raised total IgE level). Any previous exposure to *H. pylori* infection up to age 6.5 was significantly associated with a 46% decreased odds of atopy. We also explored the effects of CagA+ strains of *H. pylori* infection for the first time in this cohort at the age 6.5-year follow-up visit. Infection with a CagA+ *H. pylori* strain was associated with a 65% decreased risk of atopy, and reached borderline statistical significance. In longitudinal analyses, infection with *H. pylori* at age 3 was associated with a 51% decreased risk of incident atopy at age 6.5 years.

We do not think that our findings result from selection bias for several reasons. First, the study cohort maintained high follow-up proportions throughout the entire study period: 85.1% of the original cohort at birth, and 99.3% of those available at year 5 provided data at subsequent follow-up. Secondly, children who had IgE measurement results did not differ with regard to environmental, demographic and life style variables from those who had no IgE measurements. We also used a highly sensitive and specific *H. pylori* stool antigen test (49). In addition, the key outcome (atopy) was defined using age-specific total IgE cutoffs, though the sensitivity and specificity of serum total IgE determination still remains controversial (50, 51). With normal values differing in their range, an upper limit of 144 IU/ml for total IgE levels in children is generally accepted to distinguish atopics from non-atopics (51).

The role of total IgE as a biomarker for atopy and allergic disease has been suggested in several studies (39, 51-53). Two cross-sectional studies by Burrows *et al* (39) and Sunyer *et al* (52), reported an independent effect of total IgE on asthma using the data from Tucson and the European Community Respiratory Health Survey (ECRHS) study, respectively. Others demonstrated a strong positive relationship between markers of the atopic syndrome and serum total IgE levels in children (53), and suggested total IgE as a tool to discriminate those with and without atopy (51).

Despite these observations, however, elevated IgE levels are occasionally observed in disorders like parasitic infection (48), myeloma (54), and chronic inflammatory bowel diseases (55). Even though these conditions remain a possibility, in this cohort all study participants were apparently healthy children, and the magnitude of intestinal parasitosis was reported very low at age 3 (17). This suggests the role of intestinal parasites in modulating the immune system and increasing serum IgE levels is likely to be low in this study cohort.

In this study, the exposure variable representing current *H. pylori* infection (being positive for stool antigen test) at age 6.5 was inversely, though not significantly, related to prevalence of atopy (defined as raised total IgE level). Any previous exposure up to age 6.5 (suggestive of early age exposure), was significantly associated with a 46% decreased risk of atopy (defined as raised total IgE level). Other studies have also shown no cross-sectional associations between *H. pylori* infection and raised total IgE among older children (age 7.1-15.0 years) in Finland (56), and in young adults in Germany and Spain (57); the latter study reported a similar magnitude of effect estimate as our study (OR =0.88, vs. 0.87 in the current study) (57). Failure to detect a significant protective effect of infections on the risk of atopy and allergic disease at age 6.5 might be related to the age of the population being studied and/or the time of infection. Early life infection is considered to have a stronger protective effect due to the fact that programming of Th-cell memory against allergens commonly occurs during early childhood (58); the critical time period during which immunomodulation with long-lasting effects is considered most successful, probably even within the first 2 years of life (59). Thus, infections occurring later in childhood, may have shifted risk estimates towards no effect among the children studied, i.e., could have masked potentially protective effects. A study in Japan reported strong inverse associations between *H. pylori* infection and serum IgE level in younger than older adults (60).

Similar patterns of significant inverse associations between any previous *H. pylori* infection up to age 6.5, but not at age 6.5, with an outcome variable 'any allergic condition' (defined as a positive response to one or more of asthma, hay fever and eczema) also fit with previous observations from a large population based study in the United States, by Chen and Blaser (15), who used data from the 1999-2000 *National Health and Nutrition Examination Survey* (NHANES), and stratified their analysis according to participants age (3–19 years). This revealed a strong inverse association between *H. pylori* infection and onset of asthma among children age 2-4 years old (OR: 0.32), but this inverse association became weaker and non-significant when the analysis was done among children age greater than 5 years (OR:0. 74).

The findings of this study must, however, be interpreted with caution. Although the atopy outcome was measured objectively using serum IgE, measures of wheeze, eczema, hayfever and asthma were based on maternal questionnaire reporting, and hence are susceptible to misclassification and recall bias. The questionnaire was based on the widely used and validated ISAAC symptoms questionnaire (44) that has been successfully used in previous studies in adults in Butajira (61), in under-five children (62, 63) and in older age groups in Jimma, Ethiopia (8, 64), increasing the validity of our findings.

This study also provides a borderline significant inverse association between colonization with *H. pylori* CagA+ strains and atopy (defined as raised total IgE) at age 6.5 (Adjusted OR=0.35, 95%CI, 0.12 to 1.01, p=0.051). Most of the available observational studies did not investigate the role of CagA strains on allergic disorders in general, and on atopy (defined as raised total IgE) in particular. Only one study from the US National Health and Nutrition Examination Survey (23) reported an inverse association between colonization with *H. pylori* CagA+ strains and skin sensitization to pollen and moulds (as measured by SPT).

Although it may be difficult to compare the current finding due to the difference in outcome measurements (Total IgE vs. SPT to specific allergens), previous studies indicated a good correlation between an elevated total IgE and SPT in predicting atopy in children (65, 66). Comparing SPT to total serum IgE levels, Fajraoui *et al.* found that the two tests agree in 80% of cases (67). Matricardi *et al.* followed total and allergen- specific IgE levels in children from birth to 13 years of age (68). This study revealed that the evolution of total IgE was extremely heterogeneous but was parallel with that of allergen-specific IgE from the age of 5 onwards.

The hypothesis that more virulent CagA+ strains of *H. pylori*, may have a stronger protective effects against asthma and allergic diseases has been reported in a few epidemiological and animal studies (23, 24, 26). Although we found a borderline significant association in our study, the magnitude and direction of observed effect estimate was in the expected pattern, and our analyses were limited by insufficient statistical power as a result of the low prevalence of *H. pylori* infection at age 6.5 years. The low prevalence of *H. pylori* infection at the age 6.5 follow-up visit in this cohort may be linked to spontaneous elimination of the bacterium (69), or better attention to health issues in older children, or use of antibiotics for other common diseases (70). Further study is warranted to understand the exact reason of declining *H. pylori* prevalence in older children in Ethiopia.

In conclusion, our study among young children from a low-income birth cohort provides further evidence that any exposure of *H. pylori* up to age 6.5, suggestive of early colonization, is inversely associated with atopy and any allergic condition. A modest inverse association with atopy was observed among children infected with the more virulent CagA+ strains of *H. pylori*.

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Competing interests

We declare that we do not have any conflicts of interest.

Authors' contributions

BT conceived and designed the study and collected data in the field and performed data analysis, and drafted the manuscript. AA and GM participated in data collection, assisted with the design, performed analysis, interpretation of data and the critical review of the manuscript. GD, FE, AT, KR and AF participated in study design and interpretation of data, helped in drafting the manuscript and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Fig. 1 The study cohort

Supporting Information

Table 1. Prevalence of self-reported allergic symptoms and atopy at age 6.5 years according to place of residence, Butajira Birth Cohort Study, Ethiopia.

	Place of residence				
Variables	**Overall N(%)**	**Urban n(%)**	**Rural n(%)**	**Crude OR¥¥¥ (95 % CI)**	**P-value**
Wheeze (N= 806)	18(2.2)	2(2.1)	16(2.3)	0.92(0.21 to 4.08)	0.92
Eczema (N= 806)	4(0.5)	1(1.0)	3(0.4)	2.45(0.26 to 24.1)	0.43
Hay-fever (N= 806)	4(0.5)	0(0.0)	4(0.6)	NA	NA
Any allergic condition¥ (N=806)	24(3.0)	3(3.1)	21(3.0)	1.06(0.31 to 3.62)	0.93
Atopy ¥¥ (N=713)	129(18.1)	6(6.1)	123(20.0)	0.26(0.11 to 0.60)	<0.01

¥Any allergic condition' was defined as a positive response either to wheeze, or eczema, or hay fever in the past 12 months, at years 6.5 follow-up

¥¥ Atopy was defined as total serum IgE level > 144IU/ml (according to the manufacturer's recommended threshold by age)

¥¥¥ Crude odds ratio (OR) was calculated using binary logistic regression

Table 2. Associations between Atopy at age 6.5 years and *H. pylori* exposures from age 3-6.5 years, Butajira birth cohort study, Ethiopia. Cross sectional analysis (N=713).

	Atopy at age 6.5 years¥						
Variables	**Overall N(%)**	**Yes n(%)**	**No n(%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR¥¥¥¥ (95 % CI)**	**P-value**
Exposure to *H. pylori* at age 6.5							
No	634(88.9)	118(18.6)	516(81.4)	1		1	
Yes	79 (11.1)	12(15.2)	67(84.8)	0.78(0.41-1.49)	0.46	0.87(0.45 to 1.70)	0.69
Exposure to *H. pylori* up to age 6.5¥¥ (N=560)							
Never infected	165(29.5)	32(19.4)	133(80.6)	1		1	
Infected at any age up to age 6.5	393(70.5)	43(10.9)	352(89.1)	0.51(0.31-0.84)	<0.01	0.54(0.32 to 0.92)	0.02
Persistent exposure of *H. pylori*¥¥¥							
No	467(95.3)	72(15.4)	395(84.6)	1		1	
Yes	23(4.7)	3(13.0)	20(87.0)	0.82(0.24-2.84)	0.76	0.93(0.26 to 3.39)	0.92

¥ Atopy was defined as total serum IgE level > 144 IU/ml (according to the manufacturer's recommended threshold by age)

¥¥ *Exposure of H pylori infection at any age from 3-6.5 years*

¥¥¥ *H. pylori positive* at both time points (3.5 & 6.5 years of age)

¥¥¥¥ *Adjusted for area of residence, history of maternal allergy, number of siblings and maternal education using multiple logistic regression analysis.*

Table 3. Associations between allergic conditions at age 6.5 years and *H. pylori* exposure from age 3-6 years, Butajira birth cohort study, Ethiopia. Cross sectional analysis

	Any allergic conditions at age 6.5 years¥						
Variables	**Overall N (%)**	**Yes N (%)**	**No N (%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR¥¥¥¥ (95 % CI)**	**P-value**
Exposure to *H. pylori* at age 6.5 (N=797)							
No	716(89.8)	21(2.9)	695(97.1)	1		1	
Yes	81(10.2)	1 (1.2)	80(98.8)	0.41(0.06 to 3.12)	0.39	NA	NA
Exposure to *H. pylori* up to age 6.5¥¥ (N=559)*							
Never infected	194(34.7)	9(4.6)	185(95.4)	1		1	
Infected at any age up to age 6.5	365(65.3)	5(1.4)	360(98.6)	0.29(0.09 to 0.86)	0.03	0.31 (0.10 to 0.94)	0.04
Persistent exposure to *H. pylori*¥¥¥ (N=559)							
No	537(96.1)	12(2.2)	525(97.8)	1		NA	
Yes	22(3.9)	1(4.5)	21(95.5)	1.98(0.25 to 15.98)			

¥*'Any allergic condition' was defined as a positive response to one or more of asthma, hay fever and eczema in the past 12 months, at years 6.5 follow-up*

¥¥ Exposure *to H. pylori infection at any age from 3-6.5 years*

¥¥¥ *H. pylori positive* at both time points (3.5 and 6.5 years of age)

¥¥¥¥ *Adjusted for area of residence, maternal education and household size using multiple logistic regression analysis.*

Table 4. Associations between atopy at age 6.5 years and CagA *H. pylori* exposure at 6.5 years, Butajira birth cohort study, Ethiopia. Cross sectional analysis

	Atopy at age 6.5 years [a]						
Variables	**Overall N (%)**	**Yes N (%)**	**No N (%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR**[e] **(95 % CI)**	**P-value**
***H. pylori* CagA status** [b]							
Negative	629(88.2)	121(19.2)	508(80.8)	1	-	1	-
[c]***H.pylori +/ CagA***	31(4.3)	4 (12.9)	27(87.1)	0.62(0.21 to 1.81)	0.38	0.63(0.21 to 1.87)	0.63
[d] ***CagA +***	53(7.4)	4(7.5)	49(92.5)	0.34 (0.12 to 0.96)	0.04	0.35(0.12 to 1.01)	0.051

[a] Atopy was defined as total serum IgE level : > 144 IU/ml (according to the manufacturer's recommended threshold by age)

[b] Exposure *of CagA strain H. pylori infection at 6.5 years,* [c] *H. pylori* positive but CagA strain negative. [d] *Positive for both H. pylori and* CagA *strain*

[e] *Adjusted for place of residence, history of maternal allergy, and number of older sibling, , using multivariate logistic regression analysis.*

Table 5. Associations between **allergic conditions** at age 6.5 years and CagA *H. pylori* exposure at 6.5 years, Butajira birth cohort study, Ethiopia. Cross sectional analysis (N=799)

	Allergic conditions at age 6.5 years¥						
Variables	**Overall N (%)**	**Yes N (%)**	**No N (%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR¥¥¥ (95 % CI)**	**P-value**
***H. pylori* CagA status ¥¥**							
Negative	716(88)	21(2.9)	695(97.1)	1	-	1	-
H. ***pylori + /CagA -***	34(4.1)	2 (5.9)	32(94.1)	2.07(0.46 to 9.21)	0.34	2.01 (0.45 to 9.07)	0.36
CagA +	49(6.1)	1 (2.0)	48(98.0)	0.69 (0.09 to 5.23)	0.72	0.73(0.10 to 5.56)	0.76

¥*'Any allergic condition' was defined as a positive response to one or more of asthma, hay fever and eczema in the past 12 months, at yeas 6.5 follow-up*

¥¥ Exposure to *CagA strain H. pylori infection at 6.5 years*

¥¥¥ *Adjusted for sex, place of residence, number of older siblings and, history of maternal and paternal allergy, using multivariate logistic regression analysis.*

Table 6. Models for longitudinal association between atopy at age 6.5 and exposure to *H. pylori* infection at age 3 years, Butajira birth cohort study, Ethiopia (N=444)

	Atopy incident at age 6.5 years¥						
Variables	**Overall N (%)**	**Yes N (%)**	**No N (%)**	**Crude OR (95 % CI)**	**P-value**	**Adjusted OR¥¥¥ (95 % CI)**	**P-value**
Exposure to *H. pylori* at age 3 (N=444) ¥¥							
No	256(57.7)	48(18.8)	208(81.2)	1		1	
Yes	188(42.3)	18(9.6)	170(90.4)	0.46(0.26 to 0.82)	<0.01	0.49(0.27 to 0.89)	0.02

¥ *Atopy was defined as total serum IgE level > 144 IU/ml (according to the manufacturer's recommended threshold by age)*

¥¥ Exposure to *H. pylori infection at age 3 years*

¥¥¥ Adjusted for gender, area of residence, and number of siblings using multiple logistic regression analysis.

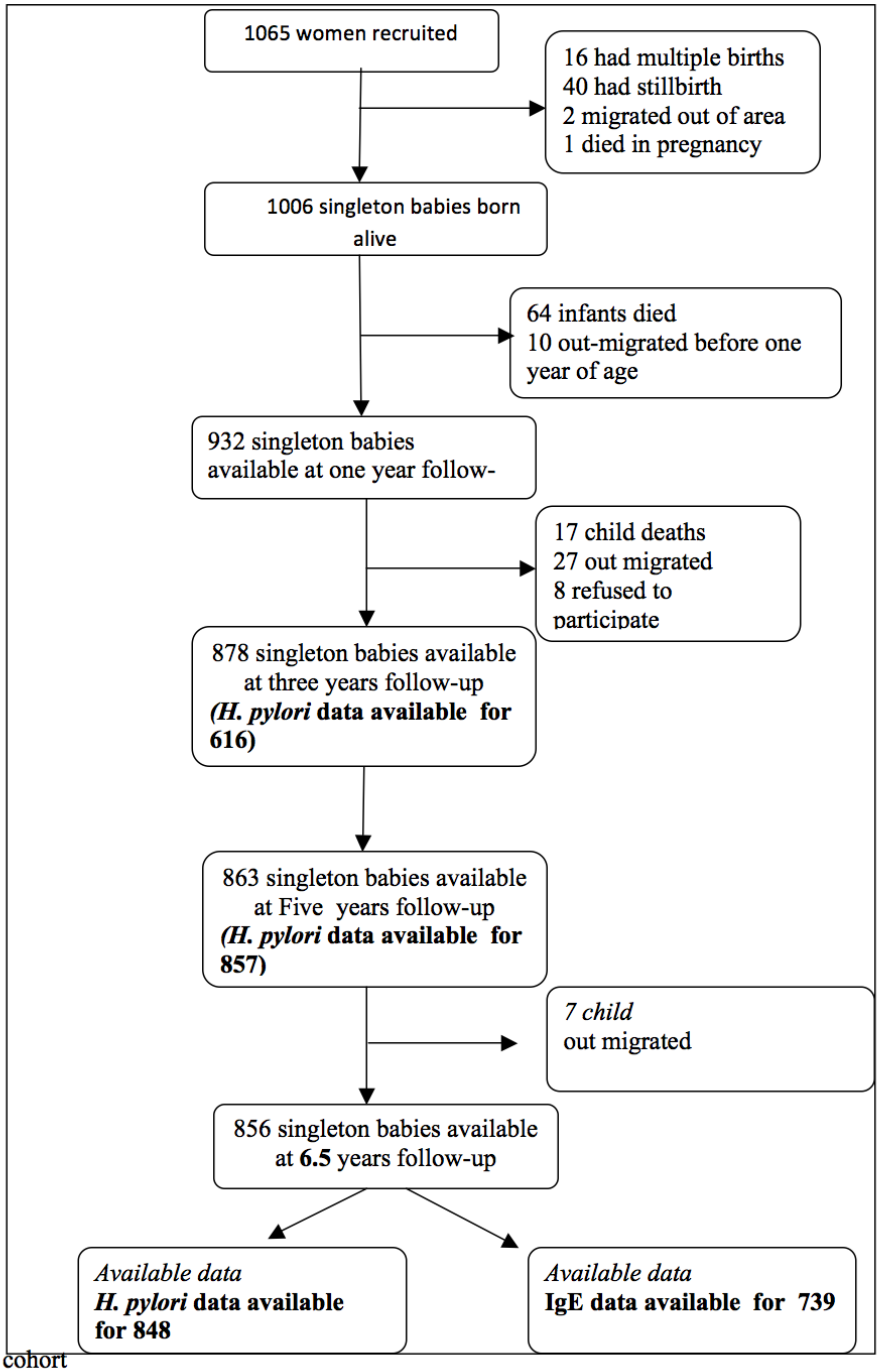

Fig. 1 The study cohort