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Original article

Anti-*Ascaris* immunoglobulin E associated with bronchial hyper-reactivity in 9-year-old rural Bangladeshi children

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IgE, Immunoglobulin E; BA, Bronchial asthma; Th1 immunity, T-helper 1 immunity; Treg, T regulatory; IL-10, Interleukin-10; TGF, Transforming growth factor; OR, Odds ratio; BHR, Bronchial hyper-responsiveness; ISAAC, International Study of Asthma and Allergies in Childhood; FEIA, Fluoro enzyme immunoassay; DP, *Dermatophagoides pteronyssinus*; FVC, Forced vital capacity; FEV_1, Forced expiratory volume in one second; SD, Standard deviation

ABSTRACT

Background: Studies have addressed the immunomodulatory effects of helminths and their protective effects upon asthma. However, anti-*Ascaris* IgE has been reported to be associated with an increased risk of asthma symptoms. We examined the association between serum levels of anti-*Ascaris* IgE and bronchial hyper-responsiveness (BHR) in children living in rural Bangladesh.
Methods: Serum anti-*Ascaris* IgE level was measured and the BHR test done in 158 children aged 9 years selected randomly from a general population of 1705 in the Matlab Health and Demographic Surveillance Area of the International Centre for Diarrhoeal Disease Research, Bangladesh. We investigated wheezing symptoms using a questionnaire from the International Study of Asthma and Allergies in Childhood. BHR tests were successfully done on 152 children (108 'current wheezers'; 44 'never-wheezers'). We examined the association between anti-*Ascaris* IgE level and wheezing and BHR using multiple logistic regression analyses.
Results: Of 108 current-wheezers, 59 were BHR-positive; of 44 never-wheezers, 32 were BHR-negative. Mean anti-*Ascaris* IgE levels were significantly higher (12.51 U_A/ml; 95% confidence interval (CI), 9.21–17.00) in children with current wheezing with BHR-positive than in those of never-wheezers with BHR-negative (3.89; 2.65–5.70; *t* test, $p < 0.001$). A BHR-positive test was independently associated with anti-*Ascaris* IgE levels with an odds ratio (OR) = 7.30 [95% CI, 2.28–23.33], $p = 0.001$ when adjusted for total IgE, anti-*Dermatophagoides pteronyssinus* IgE, pneumonia history, parental asthma, *Trichuris* infection, forced expiratory volume in one second, eosinophilic leukocyte count, and sex.
Conclusions: Anti-*Ascaris* IgE level is associated with an increased risk of BHR among 9-year-old rural Bangladeshi children.

Introduction

The specific features of bronchial asthma (BA) are chronic inflammation and hyper-responsiveness of the airways. BA is one of

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the most common chronic diseases of childhood.[1,2] A sharp rise in the worldwide prevalence of BA since the 1970s has been documented, and certain environmental factors have been postulated to contribute to this increase.[3] Such findings provoked the hypothesis that helminthic infections, which are common in rural and 'developing' areas, might protect against asthma and allergy.

The 'hygiene hypothesis', first proposed by Strachan,[4] accounts for the increase in terms of the balance between T-helper 1 (Th1) and Th2 immunity. However, the 'modified hygiene hypothesis' explains the inverse association by invoking the notion that a downregulatory mechanism (now recognized as a T regulatory (Treg) immune response) works in the presence of chronic infection. Specifically, the modified hygiene hypothesis suggests that T-regulatory cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)-β are stimulated if there is a chronic infection regardless of the type of T-helper cell, and suppress the inflammatory process.[5] This downregulation is more effective in suppressing asthma and allergy in 'developing' countries where hygiene is poor and there is a greater probability of being exposed to infectious microbes than in 'developed' countries.[6]

Epidemiological studies have examined whether helminthic infections increase or decrease the prevalence of asthma and allergy, but the results have been controversial. However, it seems likely that infection by *Ascaris* increases the prevalence of wheezing. Treatment of *Ascaris* infection has been shown to decrease the severity of asthma in Venezuela.[7] A systematic review and meta-analysis of 22 studies calculated the odds ratio (OR) of *Ascaris* infection for wheezing to be 1.34.[8] A study undertaken in China of bronchial hyper-responsiveness (BHR), which is a more specific indicator of asthma symptoms than simple recurrent wheezing, showed that *Ascaris* infection increased the prevalence of BHR.[9] It has been suggested that non-atopic asthma is associated with *Ascaris* infections in children living in poor neighborhoods in Brazil.[10] Furthermore, anti-*Ascaris* immunoglobulin (Ig)E seems to contribute to the development of wheezing. High levels of anti-*Ascaris* IgE have been found to be associated with an increased risk of asthma in a cohort study from the former East Germany where exposure to *Ascaris* was low.[11] A study of a population in South Africa exposed to mild infection by *Ascaris* suggested that anti-*Ascaris* IgE was a risk factor for atopic diseases.[12] Anti-*Ascaris* IgE has been shown to increase the risk of BHR in Costa Rica.[13] In addition to the observation that anti-*Ascaris* IgE increases the risk of wheezing in low endemic areas, we demonstrated that elevated levels of anti-*Ascaris* IgE were associated with wheezing in children in rural Bangladesh, where the prevalence of *Ascaris* infection reached ≥75%.[14] Anti-*Ascaris* IgE was shown to be a risk factor for wheezing and/or atopy in preschool-aged Brazilian children.[15] The findings mentioned above suggest that anti-*Ascaris* IgE is associated with an increased risk of wheezing regardless of exposure levels to *Ascaris*.

However, our former study was based on a questionnaire rather than on direct measurements of BHR. In the present study, we included BHR testing to ascertain if anti-*Ascaris* IgE levels are associated with BHR in high endemic areas. This strategy enabled us to dissect the results of the 2001 study.

Methods

Ethics, consent and permissions

The study protocol the Addendum of the protocol #2000-038 was approved by the Ethical Review Committee of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). This study involves human subjects and it meets the ethical principles of the Declaration of Helsinki. We also obtained written informed consent from the guardians of the children who participated in the study.

Study site and participants

The study population was from Matlab, a riverine rural area located 45 km southeast of Dhaka, the capital of Bangladesh, as described previously.[14,16] We had identified 219 children who had experienced wheezing during the previous 12 months ('current wheezers'), and randomly selected 122 controls who had never wheezed ('never-wheezers') using a questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC) in the study conducted in 2001. We administered this questionnaire again to these children in 2005. We found that 114 children out of the 194 former current wheezers whose guardians provided written informed consent reported 'current wheezing'. For comparison, we randomly selected 82 never-wheezers from the 122 never-wheezers, and 69 responded to the questionnaire after their guardians provided written informed consent. Out of these children, we found 44 never-wheezers in 2005 (Fig. 1). We undertook a BHR test on these children in 2005. There were several reasons for non-participation: five children left the area; 20 refused to participate; five were absent at the time of the visit; eight were too ill to participate.

Data collection

Collection of data and samples was undertaken from March to July 2005. Children were referred to Matlab Hospital if they agreed to participate in the study. At the hospital, study physicians took a medical history and ascertained the physical status of the children, and carried out a BHR test after obtaining written informed consent from their guardians. Data from the ISAAC questionnaire and data on other socio-environmental factors were obtained.[17] Serum samples were separated within 3 h of blood collection and stored at −20 °C. Total level of IgE in serum was measured using the fluoro enzyme immunoassay (FEIA) method (Pharmacia KK, Tokyo, Japan). IgE antibodies specific to *Dermatophagoides pteronyssinus* (DP) and *Ascaris lumbricoides* were measured by the CAP-FEIA system (Pharmacia KK). Fresh stool specimens were examined for parasites using a direct smear method without concentration.[14]

BHR testing

A bronchial challenge test that complied with the ISAAC protocol with some modifications was carried out.[18–20] Microsoft Windows™-based spirometer software (Spiro 2000) was used to measure the forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). Hypertonic saline (4.5%) was used for challenge with an ultrasonic nebuliser (U17; Omron Healthcare, Kyoto, Japan). This nebuliser could produce an output of ≥1.5 ml/min and generate an aerosol with a mass median diameter of 2–5 μm. Salbutamol, cromoglycate, theophylline and anti-histamine agents were withheld before the airway-challenge test according to the protocol. In accordance with the ISAAC protocol, provocation tests were not carried out on children with a FEV_1 <75% of the predicted value. In such cases a bronchodilator was administered and repeated spirometry done 10-min later.

The exposure time for inhaling 4.5% hypertonic saline was increased progressively from an initial exposure time of 30 s to 1, 2, 4 and 8 min. FEV_1 was measured after each exposure. The challenge was stopped if the fall in FEV_1 was ≥15% or after a maximum inhalation period of 15.5 min. A positive BHR test was defined as a fall by ≥15% of the baseline value in FEV_1, or FEV_1 of <75% of the predicted value with recovery by bronchodilators.

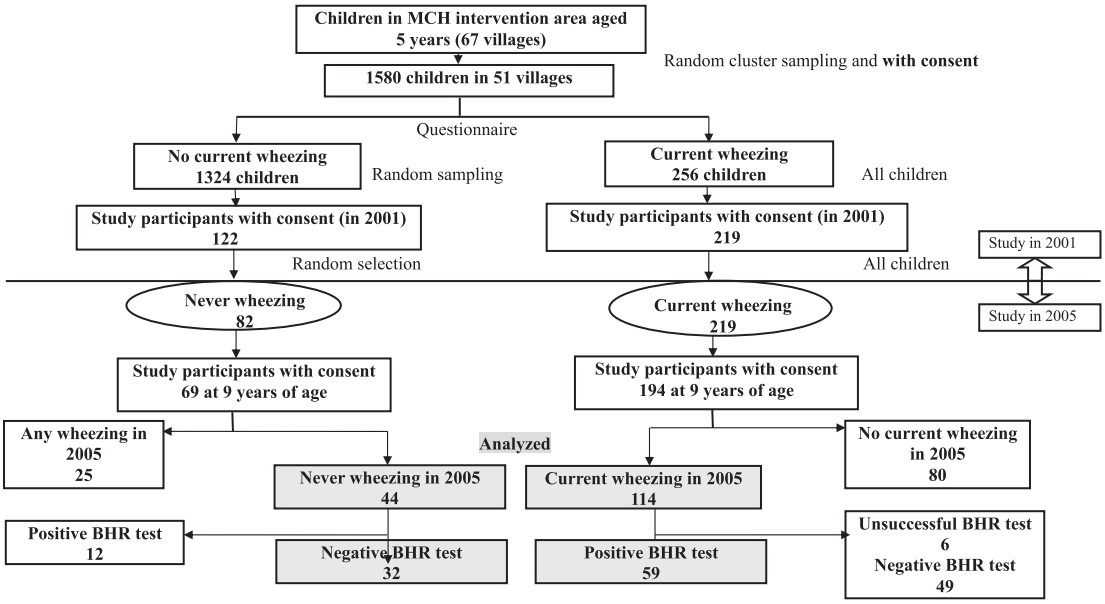

Fig. 1. Flowchart of the sampling procedure. In the study conducted in 2001, villagers were collected randomly from all 67 villages in the DSS intervention area until the total number of included children of the targeted age exceeded 1700 (the calculated sample size). Finally, 1705 children were selected from 51 of the villages and 1580 children participated in the study with written informed consent. Finally, participants comprised 219 'current wheezers' and 122 'never-wheezers'. In the 2005 study, we approached all the wheezing children and randomly selected 82 never-wheezing children, who at that time were 9 years of age. Of the 219 wheezing children, 194 agreed to participate, and 69 of the 82 never-wheezing children agreed. We found 114 current-wheezing children in the 194 former-wheezing participants and 44 never-wheezing children in the 69 former never-wheezing participants. We undertook lung function tests on these 158 children. Out of the 114 current-wheezing children, 59 showed a BHR-positive test and 32 were BHR-negative out of the 44 never-wheezing children. Analyses were done first between the 114 current wheezing children and 44 never-wheezing children, and then between the 59 BHR-positive children and 32 BHR-negative children.

Statistical analyses

Sample size was calculated according to the mean and standard deviation (SD) values of $\log_e$-transformed anti-*Ascaris* IgE values among current wheezers and never-wheezers, which were 2.78 U_A/ml (SD, 1.37) and 2.07 U_A/ml (SD, 1.52), respectively.[14] To determine the difference between BHR-positive and BHR-negative groups with 80% power at a significance level of 5%, we estimated that we required a sample size of 21 subjects per group. To obtain this number of BHR-positive subjects, we tested 42 children in each wheezing group because the sensitivity of the challenge test using 4.5% saline was 30–50%.

Data were analysed using SPSS v22 (IBM Japan, Tokyo, Japan). Analyses were undertaken first between the consistently wheezing group (n = 114) and the consistently never-wheezing group (n = 44). Then, analyses were carried out between the BHR-positive children in the current wheezers (n = 59) and the BHR-negative children from the never-wheezers (n = 32). After each variable had undergone descriptive analyses, crude ORs and adjusted ORs of IgE values for consistently current wheezers and BHR results were calculated using logistic regression models. Odds ratio (OR) of anti-*Ascaris* IgE was adjusted for sex, pneumonia, parental asthma, *Trichuris* infection, eosinophils, total IgE, anti-DP IgE and FEV_1. OR of total IgE was adjusted for sex, pneumonia, parental asthma, *Trichuris* infection, eosinophils, anti-*Ascaris* IgE, anti-DP IgE and FEV_1. OR of anti-DP IgE was adjusted for sex, pneumonia, parental asthma, *Trichuris* infection, eosinophils, anti-*Ascaris* IgE, total IgE and FEV_1. OR of FEV_1 was adjusted for sex, pneumonia, parental asthma, *Trichuris* infection, eosinophils, anti-*Ascaris* IgE, total IgE and anti-DP IgE. Although in the previous study in the year 2001 there was a weak tendency that children with short stature and lower weight and from lower income household were more likely to wheeze, the result was not always consistent. Thus, we did not include these variables as co-variates.

There were 10 children who were non-wheezers in 2001 but current wheezers in 2005, and 9 children who were current wheezers in 2001 but non-wheezers in 2005. We compared the anti-*Ascaris* IgE and anti-DP IgE levels and the prevalence of BHR positive rates between these 2 groups.

Results

Among the selected 301 children, 263 (87%) children agreed to participate in the study (Fig. 1). We found 114 children who reported current wheezing again in 2005 from the 194 current wheezing children in 2001 and 44 never-wheezing children from the 69 never-wheezing children in 2001. The BHR test was carried out on these children. Among 158 BHR-tested participants, 152 children were challenged successfully. Out of 114 wheezing children, 59 (55%) were BHR-positive, and 32 (73%) were BHR-negative out of 44 never-wheezing children.

Then, we first compared the characteristics of participants between the 114 wheezing group and 44 never-wheezing group, and second between the 59 BHR-positive with wheezing and the 32 BHR-negative without wheezing groups. Table 1 shows the variables of the study population of which the differences were proved to be significant by bivariate analysis or variables that were thought to be relevant. Significant differences between the two groups were found in the variables of: history of pneumonia; parental asthma; FVC; FEV_1.

Table 2 shows the serum levels of total IgE, anti-*Ascaris* IgE, and anti-DP IgE by the severity of asthma symptoms as expressed by the presence of: sleep disturbance; ≥4 attacks per year; speech difficulty. Anti-*Ascaris* IgE level was high in those groups as well as in the consistently wheezing group and BHR-positive group, as we reported previously.[14]

Finally, we calculated the crude OR and adjusted OR values of total and specific IgE values for wheezing and positive-BHR tests in

Table 1
Description of the characteristics of the population and the ORs of the variables used for adjustment for presence of wheezing and BHR.

		Wheezers	Never-wheezers	Crude OR	*P*	BHR positive	BHR-negative	Crude OR	*P*
		n = 114	n = 44			n = 59	n = 32		
Male/female		58/56 (51%)	23/21 (52%)	1.06 (0.53–2.12)	0.875	31/28 (52%)	14/18 (44%)	1.42 (0.60–3.38)	0.424
Helminth infection (n)†		(109)	(42)			(57)	(31)		
Ascaris	Yes	83 (76%)	29 (63%)	1.43 (0.65–3.15)	0.373	48 (84%)	24 (77%)	1.56 (0.52–4.69)	0.432
Trichuris	Yes	54 (50%)	27 (64%)	0.54 (0.26–1.14)	0.106	27 (47%)	21 (68%)	0.43 (0.17–1.07)	0.070
History of pneumonia									
During 5 years	Yes	54 (47%)	5 (11%)	7.02 (2.58–19.10)	0.001	30 (51%)	4 (13%)	7.24 (2.26–23.22)	0.001
Total eosinophils count (%)‡		11.5 (10.3–12.7)	9.6 (7.7–11.6)	1.05 (0.99–1.12)	0.102	12 (10.8–14.2)	9.5 (7.3–11.6)	1.09 (1.01–1.17)	0.037
Asthmatic parents	Yes/no	44/70 (39%)	5/39 (11%)	7.68 (1.79–33.0)	0.006	38 (64%)	4 (13%)	3.87 (1.19–12.53)	0.024
FVC‡ ml	Mean	1139 (1092–1185)	1240 (1171–1308)	0.99 (0.99–1.00)	0.025	1107 (1052–1115)	1252 (1165–1339)	0.99 (0.994–0.999)	0.008
FEV_1‡	(95% CI)	1127 (1109–1192)	1203 (1135–1271)	0.99 (0.99–1.00)	0.037	1083 (1028–1137)	1232 (1125–1311)	0.99 (0.993–0.999)	0.002

OR, odds ratio; BHR, bronchial hyper-responsiveness; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; CI, confidence interval.
† The numbers in the brackets indicate those who tested for helminth infection. Not all participants were tested.
‡ The unit of change in the calculation of OR is 1.

Table 2
Geometric mean levels of total and specific IgE.

	n	Total IgE	Anti-*Ascaris* IgE	Anti-DP IgE
		Geometric mean (95% CI) (IU/ml)	Geometric mean (95% CI) (U_A/ml)	Geometric mean (95% CI) (U_A/ml)
Never-wheezers with BHR (−)	32	1579 (1051–2372)	3.89 (2.65–5.70)	1.40 (1.09–1.80)
Never-wheezers	44	1744 (1255–2426)	4.59 (3.27–6.45)	1.42 (1.12–1.80)
Current wheezers	114	2392 (1899–3014)	9.12 (7.02–11.84)	1.60 (1.36–1.89)
Sleep disturbance	93	2119 (1650–2722)	7.94 (5.96–10.57)	1.55 (1.30–1.85)
≥4 attacks	31	2729 (1767–4219)	11.52 (7.13–18.63)	1.31 (1.06–1.63)
Speech difficulty	13	3293 (1927–5628)	13.26 (6.57–26.75)	1.50 (0.90–2.50)
BHR-positive	59	2912 (2224–3813)	12.51 (9.21–17.00)	1.92 (1.50–2.47)

IgE, immunoglobulin E; CI, confidence interval; DP, *Dermatophagoides pteronyssinus*; BHR, bronchial hyper-responsiveness.

the population with more specific asthma symptoms (Table 3). Anti-*Ascaris* IgE level proved to be associated with wheezing and a BHR-positive test in all severity groups. History of pneumonia and parental asthma were significantly and increasingly associated with wheezing and positive-BHR tests, and FEV_1 was inversely associated with wheezing and BHR.

Last when we compared the anti-*Ascaris* IgE and anti-DP IgE levels and the prevalence of BHR positive rates between the 2 groups of 10 children who were non-wheezers in 2001 but current wheezers in 2005, and of the 9 children who were current wheezers in 2001 but non-wheezers in 2005, we found no statistical difference between the 2 groups probably because of the small sample size.

Discussion

We showed that anti-*Ascaris* IgE levels were associated with an increased risk of a BHR-positive test in 9-year-old children in rural Bangladesh, where the prevalence of *Ascaris* infection was ≥75%.[14] Simultaneously, a significantly higher proportion of wheezing children in 2001 and 2005 (consistently wheezing children), who were highly likely to be suffering from BA, showed a significantly higher number of positive tests for BHR than children who were not suffering from wheezing in 2001 and 2005, and anti-*Ascaris* IgE levels were associated with an increased risk of consistent wheezing.

In Matlab helminths infections have been dramatically reduced since national mass chemotherapy strategy started around 2004. However, the participants of this study did not receive that benefit and almost 70% of both of the wheezing and non-wheezing children were infected with *Ascaris* (data not shown) at that time. Even though the prevalence became low in the younger generation in 2005, it does not necessarily mean that the prevalence in the older generation was low. There might be difference in the effect of *Ascaris* infection on wheezing between dewormed and non-dewormed groups. Recently deworming is done for children twice a year. However, it was found that soil-transmitted helminth especially *Ascaris lumbricoides* infections recur rapidly after treatment.[21] We are not sure about the difference in the extent of wheezing at the time of larval migration of *Ascaris* between re-infection and the additional infection when deworming is not done. We have to examine about this in the future.

In this study, anti-*Ascaris* IgE was associated with wheezing, but no association was observed between current infection and wheezing, suggesting that IgE reactivity, not infection itself, against *Ascaris* is related to wheezing. These findings are in agreement with those of our former study, which showed an increasing association between anti-*Ascaris* IgE levels and wheezing. The present study enabled us to confirm the results that were obtained during 2001 study (which used questionnaires rather than the BHR test).

Despite reported inverse associations of helminthic infections and asthma-like symptoms in various studies, *Ascaris* seems to be positively associated with asthma-like symptoms.[7–15] In addition, Hagel *et al.* reported that anti-*Ascaris* IgE level is associated with an increased risk of BHR and decreased FEV_1 in areas where *Ascaris* is highly prevalent.[22] The study by Hagel *et al.* was the first to show an association between anti-*Ascaris* IgE level and BHR in highly endemic areas, but it did not take other confounding factors into consideration. Associations between BHR and anti-*Ascaris* IgE level in low-prevalence areas have also been reported.[9,13] In accordance with those studies, we found a significant association between anti-*Ascaris* IgE level and BHR among children with high infectious burden.

Helminthic infections have been associated with negative skin prick tests to aeroantigens.[23] In general, helminthic infections induce a Th2 immune response characterised by production of IL-4, IL-5 and IL-13 by Th2 cells, elevated levels of IgE, tissue eosinophilia, and mastcytosis for protection of the host and,

Table 3
Odds ratios for current wheezing and BHR relative to the total, anti-*Ascaris* and anti-DP IgE including if children with less specific asthma symptoms are excluded.

	Wheezers/non-wheezers	Units of change	Crude odds ratio (95% CI)	*p*	Adjusted odds ratio (95% CI)	*p*
Anti-*Ascaris* IgE (log_e transformed)					†	
Current wheezing	114/44	1	1.46 (1.10–1.93)	0.008	2.31 (1.24–4.32)	0.009
Attack ≥4 times††	31/41	1	1.87 (1.24–2.83)	0.003	1.37 (1.18–1.96)	0.014
Sleep disturbance‡‡	93/41	1	1.38 (1.04–1.83)	0.024	2.02 (1.07–3.82)	0.031
Speech disturbance§§	14/43	1	2.18 (1.20–4.00)	0.011	4.10 (1.00–16.71)	0.049
BHR¶¶	59/32	1	2.37 (1.54–3.63)	<0.001	7.30 (2.28–23.33)	0.001
Total IgE (log_e transformed)					‡	
Current wheezing	114/44	1	1.23 (0.91–1.65)	0.178	0.56 (0.29–1.08)	0.082
Attack ≥4 times††	31/41	1	1.47 (0.96–2.26)	0.079	0.41 (0.10–1.65)	0.207
Sleep disturbance‡‡	93/41	1	1.18 (0.88–1.59)	0.279	0.58 (0.29–1.15)	0.121
Speech disturbance§§	14/43	1	1.69 (1.30–2.19)	<0.001	1.09 (0.31–3.88)	0.898
BHR¶¶	59/32	1	1.69 (1.12–2.57)	0.013	0.33 (0.11–1.01)	0.052
Anti-DP IgE (log_e transformed)					§	
Current wheezing	114/44	1	1.75 (0.87–3.50)	0.115	0.88 (0.50–1.56)	0.668
Attack ≥4 times††	31/41	1	0.96 (0.50–1.86)	0.905	0.08 (0.01–0.69)	0.022
Sleep disturbance‡‡	93/41	1	1.19 (0.74–1.90)	0.477	0.89 (0.50–1.60)	0.693
Speech disturbance§§	14/43	1	1.21 (0.99–1.48)	0.062	0.49 (0.17–1.43)	0.191
BHR¶¶	59/32	1	1.67 (0.93–3.01)	0.089	1.38 (0.66–2.88)	0.394
FEV_1					¶	
Current wheezing	114/44	1	0.998 (0.996–1.00)	0.017	0.99 (0.99–1.00)	0.194
Attack ≥4 times††	31/41	1	0.998 (0.995–1.00)	0.037	1.03 (0.998–1.05)	0.068
Sleep disturbance‡‡	93/41	1	0.998 (0.997–1.00)	0.048	1.00 (0.998–1.01)	0.179
Speech disturbance§§	14/43	1	1.11 (0.91–1.36)	0.291	0.997 (0.992–1.001)	0.167
BHR¶¶	59/32	1	0.996 (0.99–1.00)	0.001	0.995 (0.990–0.999)	0.015

CI, confidence interval; BHR, bronchial hyper-responsiveness; FEV1, forced expiratory volume in one second; DP, *Dermatophagoides pteronyssinus*.
† Odds ratio (OR) of anti-*Ascaris* IgE was adjusted for sex, pneumonia, parental asthma, *Trichuris* infection, eosinophils, total IgE, anti-DP IgE and FEV_1.
‡ OR of total IgE was adjusted for sex, pneumonia, parental asthma, *Trichuris* infection, eosinophils, anti-*Ascaris* IgE, anti-DP IgE and FEV_1.
§ OR of anti-DP IgE was adjusted for sex, pneumonia, parental asthma, *Trichuris* infection, eosinophils, anti-*Ascaris* IgE, total IgE and FEV_1.
¶ OR of FEV_1 was adjusted for sex, pneumonia, parental asthma, *Trichuris* infection, eosinophils, anti-*Ascaris* IgE, total IgE and anti-DP IgE.
†† Individuals who had experienced fewer than four attacks of wheezing during the previous year were excluded from the analyses.
‡‡ Those whose sleep had not been disturbed during the previous year were excluded from the analyses.
§§ Those whose speech had not been disturbed by wheezing during the previous year were excluded from the analyses.
¶¶ Dependent variable was BHR-positive or not.

simultaneously, they stimulate Treg cells and production of cytokines such as IL-10 and TGF-beta.[24–26] This concept has been studied comprehensively in filariasis.[27,28] Treg-type immunity is considered to play a crucial part in suppressing asthma symptoms in developing countries, where the infectious burden because of helminths is considerable.[5,6,29]

Despite these observations suggesting that helminths suppress allergy, anti-*Ascaris* IgE seems to contribute to the enhancement of asthma-like symptoms.[11–15] However, the role of anti-*Ascaris* IgE in asthma symptoms is not obvious, and may be fourfold.

First, anti-*Ascaris* IgE may act like an antibody to inhalant antigens to trigger degranulation of mast cells to induce Th2 inflammation on re-exposure to inhalant *Ascaris* antigen. Second, the elevated anti-*Ascaris* IgE level might only be coincident with larval migration after infection. *Ascaris* nematodes migrate through the lungs during maturation, and cause Th2-type pulmonary inflammation and episodic wheezing. In such a situation, anti-*Ascaris* IgE production would be boosted by re-exposure to *Ascaris* antigen. We might merely be observing coincident enhanced production of anti-*Ascaris* IgE and asthma-like symptoms, which is well known as 'tropical pulmonary eosinophilia'. Third, although we speculate that anti-*Ascaris* IgE causes wheezing/BHR, the higher anti-*Ascaris* IgE levels observed in the wheezing/BHR group might be because atopic children produce more anti-*Ascaris* IgE in response to stimulation by *Ascaris*.[13] In the present study, however, the anti-*Ascaris* IgE level retained its significance after adjustment for anti-DP IgE, suggesting that it is an independent risk factor for wheezing/BHR.[14] Fourth, although various reports have attributed involvement of anti-*Ascaris* IgE to cross-reactivity with IgE from mites or cockroaches, the magnitude of these IgE antibodies bore no comparison with that of anti-*Ascaris* IgE in our previous and present study.[14] Thus, we speculate the presence of a different mechanism in the effect of anti-*Ascaris* IgE on the development of wheezing/BHR other than cross-reactivity with the IgE of mites or cockroaches.

Other risk factors for a BHR-positive test were found to be a history of pneumonia, total IgE, parental asthma, and FEV_1. A history of pneumonia has remained a risk factor consistently throughout our studies. This observation is in agreement with the consensus regarding development of recurrent wheezing in children worldwide. FVC and FEV_1 had inverse associations with wheezing and BHR (Table 1). However, we omitted FVC from the analyses because of multi-collinearity: the variance inflation factor was >10. Although we measured FEV_1/FVC, it had no association with wheezing or a BHR-positive test (data not shown). This result may have been because of our lack of technical skill for measuring FVC, or insufficient expiration at the time of measurement. In our previous study in 2001 there was a weak association between short stature and low weight. A study from Matlab also reported the association of wheezing, although this study did not take respiratory tract infections, the strongest risk factor for wheezing, into consideration on.[30]

Our study did not indicate any contribution of *Ascaris* infection to wheezing nor BHR positivity. However, the effect of the severity of *Ascaris* infection (as measured by egg counts) to the development of asthma symptoms has been reported from the same rural area of Bangladesh (Matlab) recently.[31] Studies from Matlab (including ours) imply that *Ascaris* infection *per se* or its products may have a role on the development of asthma in this area. Studies on the ability to induce a Treg response to various types of helminth (including *Ascaris*) will be required to establish their contribution on the suppression or exacerbation of asthma and allergy.[32]

The risk factors from our studies for wheezing and BHR when *Ascaris* infection is prevalent, are anti-*Ascaris* IgE, history of pneumonia and parental asthma in rural Bangladesh. *Trichuris* might be

a decreasing risk factor. When people use dry leaves for their cooking fuel, it becomes an increasing risk factor. Smoke from fuel was a risk factor because children stay with their mothers while they cook and severely were exposed. On the other hand, tobacco smoking was not a risk factor. It was probably because people smoke outside of their homes in a rural situation which does not cause passive smoking to the children.

To conclude, anti-*Ascaris* IgE plays an important role in the development of wheezing and BHR positive test among rural Bangladeshi children. Studies on the ability to induce a Treg response to various types of helminth (including *Ascaris*) will be required to establish their contribution on the suppression or exacerbation of asthma and allergy.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

HT designed the study, contributed to data collection, data analysis and interpretation of results, and has written the manuscript. AFK and MY designed the study, contributed to data collection. MIH and MDHH contributed to data collection, and have critically revised the manuscript. ST and HK contributed to data collection, data analysis and interpretation of results. KZ designed the study, and has critically revised the manuscript. HRC contributed to data collection. YW designed the study, and has critically revised the manuscript. SN has written and critically revised the manuscript. TI designed the study, contributed to interpretation of results, and has critically revised the manuscript. All the authors approved the final manuscript.

References

1. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000;**161**:1720–45.
2. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;**320**:271–7.
3. von Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994;**149**:358–64.
4. Strachan DP. Hay fever, hygiene, and household size. *Br Med J* 1989;**299**:1259–60.
5. Yazdanbakhsh M, Matricardi PM. Parasites and the hygiene hypothesis: regulating the immune system? *Clin Rev Allergy Immunol* 2004;**26**:15–24.
6. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002;**296**:490–4.
7. Lynch NR, Palenque M, Hagel I, DiPrisco MC. Clinical improvement of asthma after anthelminthic treatment in a tropical situation. *Am J Respir Crit Care Med* 1997;**156**:50–4.
8. Leonardi-Bee J, Pritchard D, Britton J. Asthma and current intestinal parasite infection: systematic review and meta-analysis. *Am J Respir Crit Care Med* 2006;**174**:514–23.
9. Palmer LJ, Celedon JC, Weiss ST, Wang B, Fang Z, Xu X. *Ascaris lumbricoides* infection is associated with increased risk of childhood asthma and atopy in rural China. *Am J Respir Crit Care Med* 2002;**165**:1489–93.
10. Pereira MU, Sly PD, Pitrez PM, Jones MH, Escouto D, Dias AC, et al. Nonatopic asthma is associated with helminth infections and bronchiolitis in poor children. *Eur Respir J* 2007;**29**:1154–60.
11. Dold S, Heinrich J, Wichmann HE, Wjst M. *Ascaris*-specific IgE and allergic sensitization in a cohort of school children in former East Germany. *J Allergy Clin Immunol* 1998;**102**:414–20.
12. Obihara CC, Beyers N, Gie RP, Hoekstra MO, Fincham JE, Marais BJ, et al. Respiratory atopic disease, *Ascaris*-immunoglobulin E and tuberculin testing in urban South African children. *Clin Exp Allergy* 2006;**36**:640–8.
13. Hunninghake GM, Soto-Quiros ME, Avila L, Ly NP, Liang C, Sylvia JS, et al. Sensitization to *Ascaris lumbricoides* and severity of childhood asthma in Costa Rica. *J Allergy Clin Immunol* 2007;**119**:654–61.
14. Takeuchi H, Zaman K, Takahashi J, Yunus M, Chowdhury HR, Arifeen SE, et al. High titre of anti-*Ascaris* IgE associated with bronchial asthma symptoms in 5-year-old rural Bangladeshi children. *Clin Exp Allergy* 2008;**38**:276–82.
15. Alcântara-Neves NM, Badaró SJ, dos Santos MC, Pontes-de-Carvalho L, Barreto ML. The presence of serum anti-*Ascaris lumbricoides* IgE antibodies and of *Trichuris trichiura* infection are risk factors for wheezing and/or atopy in preschool-aged Brazilian children. *Respir Res* 2010;**11**:114.
16. Zaman K, Takeuchi H, Yunus Md, El Arifeen S, Chowdhury HR, Baqui AH, et al. Asthma in rural Bangladeshi children. *Indian J Pediatr* 2007;**74**:539–43.
17. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez F, et al. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;**8**:483–91.
18. Weiland SK, Bjorksten B, Brunekreef B, Cookson WO, von Mutius E, Strachan DP. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *Eur Respir J* 2004;**24**:406–12.
19. Riedler J, Reade T, Dalton M, Holst D, Robertson C. Hypertonic saline challenge in an epidemiologic survey of asthma in children. *Am J Respir Crit Care Med* 1994;**150**:1632–9.
20. Mai XM, Nilsson L, Kjellman NI, Bjorksten B. Hypertonic saline challenge tests in the diagnosis of bronchial hyperresponsiveness and asthma in children. *Pediatr Allergy Immunol* 2002;**13**:361–7.
21. Jia TW, Melville S, Utzinger J, King CH, Zhou XN. Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. *PLoS Negl Trop Dis* 2012;**6**:e1621.
22. Hagel I, Cabrera M, Hurtado MA, Sanchez P, Puccio F, Di Prisco MC, et al. Infection by *Ascaris lumbricoides* and bronchial hyper reactivity: an outstanding association in Venezuelan school children from endemic areas. *Acta Trop* 2007;**103**:231–41.
23. van den Biggelaar AH, Lopuhaa C, van Ree R, van der Zee JS, Jans J, Hoek A, et al. The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. *Int Arch Allergy Immunol* 2001;**126**:231–8.
24. Turner JD, Faulkner H, Kamgno J, Cormont F, Van Snick J, Else KJ, et al. Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. *J Infect Dis* 2003;**188**:1768–75.
25. Fallon PG, Mangan NE. Suppression of TH2-type allergic reactions by helminth infection. *Nat Rev Immunol* 2007;**7**:220–30.
26. Anthony RM, Rutitzky LI, Urban Jr JF, Stadecker MJ, Gause WC. Protective immune mechanisms in helminth infection. *Nat Rev Immunol* 2007;**7**:975–87.
27. Mahanty S, Nutman TB. Immunoregulation in human lymphatic filariasis: the role of interleukin 10. *Parasite Immunol* 1995;**17**:385–92.
28. Babu S, Blauvelt CP, Kumaraswami V, Nutman TB. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. *J Immunol* 2006;**176**:3248–56.
29. Araujo MI, de Carvalho EM. Human schistosomiasis decreases immune responses to allergens and clinical manifestations of asthma. *Chem Immunol Allergy* 2006;**90**:29–44.
30. Hawlader MD, Noguchi E, El Arifeen S, Persson LÅ, Moore SE, Raqib R, et al. Nutritional status and childhood wheezing in rural Bangladesh. *Public Health Nutr* 2014;**17**:1570–7.
31. Hawlader MD, Ma E, Noguchi E, Itoh M, Arifeen SE, Persson LÅ, et al. *Ascaris lumbricoids* infection as a risk factor for asthma and atopy in rural Bangladeshi children. *Trop Med Health* 2014;**42**:77–85.
32. Cooper PJ. Interactions between helminth parasites and allergy. *Curr Opin Allergy Clin Immunol* 2009;**9**:29–37.