

Heightened bronchial hyperresponsiveness in the absence of heightened atopy in children with current wheezing and low-income status.

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ABSTRACT

Background: Although global studies as ISAAC have provided with valuable data on the prevalence of asthma in children of Latin America, there is very few information on the relationship between asthma symptoms, pulmonary function, bronchial hyperresponsiveness and atopy in the region. **Methods:** This study examined the relationship between self-reported wheezing in the last 12 months, pulmonary function, airway responsiveness and atopy in children from a low-income population neighborhood in Santiago, Chile. Two random samples (100 each) of children aged 13-14 years who participated in ISAAC Phase One were selected according to whether they have reported or not, wheezing in the last 12 months. Spirometry, methacholine bronchial challenge test and prick test were performed in all individuals. **Results:** Children who reported current wheezing had significantly higher bronchial hyperresponsiveness (BHR) to methacholine as compared to those without wheezing (71.6% vs.52.6%, respectively; $p=0.007$), and no significant difference was found in FEV1 ($116.7\pm 12.3\%$ vs. $120.3\pm 14.5\%$, respectively, $p=0.11$). The prevalence of atopy was not significantly different between those children who reported wheezing as compared to those who did not (44.2% vs. 42.3%; respectively, $p=0.89$). Multiple regression analysis showed that only BHR to methacholine (OR 2.72, 95% CI: 1.25-4.13, $p=0.01$) and maternal asthma (OR 3.1, 95% CI 1.2-8.3, $p=0.03$) were significant risk factors for current wheezing. **Conclusions:** Our results support previous findings suggesting that in adolescents from unprivileged populations, self-reported current wheezing is related to BHR but not to atopy.

Keywords: asthma, atopy, bronchial hyperresponsiveness, children, ISAAC.

INTRODUCTION

The largest global epidemiological study (International Study of Asthma and Allergies in Childhood, ISAAC), which includes the main regions of the world and countries with different level of development, provided with important world-wide comparative information on the prevalence of asthma symptoms in childhood. In developing regions as Latin America, this study has provided with lacking information on the prevalence of asthma revealing that apart from a wide variability in the prevalence of self reported asthma symptoms, some of its centers were within those with the highest prevalence of asthma in the world (1). It has been demonstrated that the prevalence of asthma symptoms, bronchial hyperresponsiveness (BHR) and atopy varies greatly between and within countries, independently of their development status and other cultural characteristics as language (1,2). Although the reasons for these findings are unknown, it is likely that distinct environmental factors acting at different localities could play an important role on the observed variability. BHR is considered an important feature of asthma representing a valuable objective measure for epidemiological studies on the disease. Atopy, indicated by skin reactivity to common allergens, is related with the development, prevalence, persistence and severity of asthma symptoms and also in close association with BHR in asthmatic patients and random samples from general population (3-6). In asthmatic children from developed countries, the relationship between asthma-related symptoms, lung function, BHR and atopy has been evaluated (7-10) and the findings of some population-based studies have shown an association between symptoms, atopy and BHR (11-12). However, there is some controversy with respect to whether the

deterioration of lung function and increased BHR in children with asthma symptoms are related with atopy (7-12) and doubts have been cast on the causal role of atopy in asthma (13-14). For example, Pearce et al (14), analyzing nine population-based surveys, showed that the prevalence of atopy (positive skin test) in asthmatic children from developed countries ranged from 40% to 79%, and less than 50% of asthma cases were attributable to atopy. Moreover, a large survey (15) carried out in England in adolescents and young adults demonstrated that the proportion of current wheezing attributable to atopy (house dust mite specific IgE > 0.3 kU/l) was only 35%. Recently, Garcia-Marcos et al (16) using the ISAAC questionnaire in Spanish schoolchildren showed that 62% of children with current wheezing were atopics.

In developing countries atopy would not be an important determinant for asthma symptoms or BHR in children (17-18), even when some of them are within the countries with the highest prevalence of asthma symptoms in the world (1, 19). The latter suggests that mechanisms different to atopy may be responsible for asthma symptoms and BHR in children from unprivileged areas, and perhaps for the worldwide variability reported in the prevalence of asthma, atopy and BHR (1,2,13-15). It is likely that environmental exposures, different in nature and magnitude, could determine the important variation reported in the prevalence and severity of asthma symptoms globally, and also its relationship with BHR and atopy.

The purpose of our study was to evaluate the relationships between self-reported symptoms of asthma, pulmonary function, airway responsiveness and atopy in children living in a low socioeconomic neighborhood in Santiago, Chile.

METHODS

From the 3051 schoolchildren (13 -14 years old) who participated in the official ISAAC Phase One study at our institution (1), we randomly selected 100 children who had responded "Yes" to the question "Have you had wheezing in the last 12 months?" (current wheezing group) and 100 children who responded "No" to that question (without current wheezing group). Parents were asked on the following questions: history of asthma in their first degree relatives, active tobacco smoking, environmental exposure to tobacco smoke and to other indoor pollutants at home (type of heating and cooking), pets at home (cats, dogs), type of floor covering and carpets at home and paved streets in their neighborhood.

Children were from a low-income neighborhood in Santiago i.e. monthly income per home around US\$613, 25% of population in the poverty line, mean parental educational level is 8 or less school years, and high atmospheric pollution (PM₁₀ exceeding 400 µg/m³ several times during autumn and winter).

Each subject was evaluated with spirometry, methacholine bronchial challenge and allergen skin prick test. Tests were performed in random sequence on three consecutive days. Children were studied only when they were free of respiratory symptoms for at least 4 weeks. Those subjects using asthma medication were instructed to discontinue inhaled salbutamol for 12 hours prior testing and inhaled corticosteroids were continued. None of the subjects were using oral corticosteroids, anti-histamines, or theophylline.

Lung function (spirometry) was performed using a heated Fleisch type pneumotachograph (model 3810, Hans Rudolph inc. KC., MO, US) with the Medgraphics CPF-S processing system (Medical Graphics Corp., St. Paul, MN, US). Subjects performed all maneuvers in the standing position and using nasal clip. At least 3 technically acceptable curves were obtained. Values for forced vital capacity (FVC) and FEV₁ did not differ from

the next lowest values by more than 5% or 100 mL, whichever was greater. Reported values were obtained from the best curve with the highest sum of FEV₁ and FVC.

Methacholine bronchial challenge was performed if the FEV₁ was \geq 80% of the predicted value using a modified Cockcroft's method. Methacholine chloride (ICN Biomedical Inc., Ohio, US) solution in normal saline were stored at 4°C and nebulized at room temperature using a Hudson 1730 jet nebulizer with a fill volume of 2 milliliters. The nebulizer, which was driven by air at a pressure of 344 kPa (50 psi) and flow of 6 L/min, had an output of 340 - 360 mg/min. Subjects inhaled each aerosol through a mouth tube with volume extension piece. Following the initial inhalation of normal saline, a methacholine solution of 0.03 mg/mL was inhaled by quiet mouth breathing during 2 min; FEV₁ was obtained 30 and 90 seconds after each nebulization and the lowest value was used for calculations. Doubling concentrations of methacholine were administered every 5 minutes until FEV₁ decreased by 20% from the post-saline value or a concentration of 8 mg/mL was nebulized. The provocative concentration of methacholine required to decrease FEV₁ by 20% from saline (PC20) was calculated by linear interpolation of logarithmically transformed concentrations. For the purposes of the study, BHR to methacholine was defined as PC20 < 8 mg/mL.

Skin prick test to 8 common inhalant allergens were performed on the forearm, as well as a positive (histamine) and a negative (solvent) control. The following allergens were employed: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, *alternaria*, grass mixture, trees mixture and weeds mixture (Nelco Laboratories, NY, US). Atopy was defined as a positive reaction (wheal size measuring 3mm or more after subtraction of the control value) to one or more allergens.

The Hospital Ethics Committee approved the study and full informed and signed consent was obtained from all parents.

Statistical Analysis:

The statistical analysis for differences between the two groups (current wheezing or without current wheezing) was done using Fisher and Chi-square test for categorical variables and Student t-test for continuous variables. Multivariate logistic regression was performed using current wheezing as a dependent variable; those factors that were statistically significant ($p < 0.05$) in the univariate analysis were used as independent variables.

The proportion of current wheezing cases "attributable" to BHR to methacholine was estimated by the "population attributable risk" according to Pearce et al (14). If exposure has an odds ratio for current wheezing of R, then the proportion of exposed cases attributable to exposure is expressed as $(R-1)/R$, and the proportion of all cases in the population attributable to exposure (population attributable risk) is $P(R-1)/R$, where P is the proportion of all exposed cases.

RESULTS

Ninety-five out of 100 adolescent (64 girls) in the current wheezing group and 97 out of 100 adolescent (51 girls) in the group without wheezing completed all the evaluations (spirometry, methacholine bronchial challenge and skin prick test). The main reason for failure in study completion among the 8 adolescents were: family moving out of the city (2 in the group with current wheezing and 1 in the group without wheezing) and unwilling to continue with the evaluation (3 in the group with current wheezing and 2 in the group without wheezing).

The mean (SD) age between adolescents with current wheezing and without wheezing were similar (14.53 ± 0.57 vs. 14.71 ± 0.61 years) and for height the figures were 160.6 ± 7.49 and 162.4 ± 7.39 cm, respectively. There were significantly less males in the group with current wheezing compared to that without wheezing (32.6 vs. 47.4%, $p=0.037$). Adolescents with current wheezing had higher prevalence of maternal asthma and siblings with asthma than those without wheezing (19.2% vs. 6.2%, $p=0.007$ and 18.3% vs. 7.2%, $p=0.02$, respectively), Table 1. The prevalence of active tobacco consumption (in the last 12 months) and intra-domiciliary tobacco exposure was similar between those with and without current wheezing (32.6% vs. 33.0% and 68.4% vs. 66%, respectively). Also, the prevalence of exposure to different fuels used for cooking or heating (gas, kerosene and wood), the proportion of carpets and types of floor covering at home and paved streets were similar between adolescent with and without current wheezing, Table 1.

Table 1. Demographic and environmental characteristics of adolescents with and without self-reported current wheezing.

	Current wheezing (n=95)	Without current wheezing (n=97)	OR (95 % CI)
Gender (% males) *	32.6	47.4	1.8 (1.0-3.5)
Maternal asthma (%) †	19.2	6.2	3.6 (1.3-11.6)
Paternal asthma (%)	9.7	9.3	1.1 (0.4-3.1)
Siblings with asthma (%) *	18.3	7.2	2.9 (1.1-8.6)
Tobacco active in last 12m (%)	32.6	33.0	1.0 (0.5-1.9)
Intra-domiciliary tobacco exposure (%)	68.4	66.0	1.1 (0.6-2.1)
Contaminant heating/cooking at home:			
Gas	27.4	33	0.8 (0.4-1.5)
Kerosene	70.5	65	1.3 (0.7-2.5)
Wood	8.5	5.1	1.3 (0.3-6.7)
Animals at home (stray dogs and cats)	75.8	75.3	1.0 (0.5-2.1)
Carpet at home (%)	12.6	8.3	1.6 (0.6-4.8)
Unpaved streets in neighborhood (%)	30.9	33.3	1.2 (0.6-2.5)

* $p < 0.05$, † $p < 0.01$

There was not significant difference in the proportion of positive skin test between those children who reported wheezing in the last 12 months compared with those who did not (44% vs. 42%; $p=0.89$, OR: 1.1 [95%IC: 0.6-2.0]), Table 2 and Figure 1. The prevalence of positive response to employed allergens was as follows: Dermatophagoides pteronyssinus (26.6%), Dermatophagoides farinae (20.3%), grass mixture (16.2%), cat (9.4%), dog (8.9%), weeds mixture (8.3%), alternaria (7.3%) and trees mixture (5.2%). The reported proportion of pets at home (mainly stray dogs and in less extent cats) was 76% in current wheezing children and 75% in those without wheezing. The proportion of positive

skin reaction to dog and cat was not significantly different between groups (8.4% vs. 9.3% and 12.6% vs. 6.2%, respectively).

Table 2. Pulmonary function and methacholine bronchial challenge in adolescents with and without self-reported current wheezing.

	Current wheezing (n=95)	Without current wheezing (n=97)
Baseline FVC (%predicted)	114.8 ±12.1	116.0±12.7
Baseline FEV ₁ (%predicted)	116.7±12.3	120.3±14.5
Baseline FEV ₁ /FVC (%predicted)	89.2±5.3	90.3±5.5
PC ₂₀ < 8 mg/mL (%) †	71.6	52.6
Positive skin test (%)	44.2	42.3

*p< 0.05, †p< 0.01

The baseline values of FVC and FEV₁ were similar between children with and without current wheezing (FVC: 114.78 ± 12.1 vs. 115.9 ± 12.7, respectively, p=0.50 and FEV₁: 116.72 ± 12.3 vs. 120.28 ± 14.5, p=0.07, respectively), as well as FEV₁/FCV (89.2±5.3% versus 90.3±5.5%, respectively, p=0.15), Table 2.

The group with current wheezing had a significantly larger proportion of adolescents with BHR to methacholine than the group without current wheezing (71.5% and 52.6%, respectively, p=0.007, OR: 2.3 [95%IC: 1.2-4.3]), Table 2. The proportion of current wheezing cases “attributable” to BHR to methacholine was 40%.

Among males, the proportion of them with BHR to methacholine was significantly larger in the group with current wheezing as compared to those without current wheezing (77% vs. 52%, p=0.028, OR: 3.1, [95%IC: 1-10.3]). Regarding females, the proportion of them with BHR was also larger in the group with current wheezing, however it did not reach significance (69% vs. 53%, p=0.083, OR: 2.0, [95%IC: 0.9-4.5]), Figure 1. The prevalence of positive skin test was similar in both groups (Table 2) and when stratified by gender no significant difference in the proportion of positive skin test was found between children with and without current wheezing (males: 52% and 41%, respectively, p=0.3, OR: 1.5, [95%IC= 0.5-4.2]; and females: 41% and 43%, respectively, p=0.07, OR: 0.9, [95%IC= 0.4-2.0]), Figure 1. Atopic adolescents with current wheezing had significantly more BHR to methacholine than non-atopics (negative skin test) (83.3% vs. 62.3%, p=0.024, OR: 3.0, [95%IC= 1.0-9.5]). When the groups were stratified by gender, atopic males had slightly more BHR to methacholine than non-atopics (71.4% vs. 54.8%, p=0.13, OR: 2.1 [95%IC= 0.7-6.0]) and atopic females had significantly more BHR to methacholine than non-atopics (72.9% vs. 53.7%, p=0.04, OR: 2.3 [95%IC= 1.0-5.6]).

The result of the multiple logistic regression analysis showed that BHR (adjusted OR: 2.02, [95%CI: 1.1-3.7], p=0.02) and maternal asthma (adjusted OR: 3.1, [95%CI: 1.2-8.3], p=0.03), were the only risk factors that remained significantly and independently associated with current wheezing.

DISCUSSION

This study shows that adolescents from an unprivileged urban area who reported wheezing in the last 12 months had a significantly more BHR to methacholine than those who had not current wheezing and this was not associated to atopy. Seventy-one percent of the children with current wheezing had BHR to methacholine and only 44% had atopy; a very similar proportion of atopy (42.3%) was found in those children without current wheezing but in this group the proportion of BHR was 53%.

The prevalence of atopy in children with current asthma symptoms from developed countries is relative similar, i.e. 62.4% in Spain (16), 58% in Sweden (18) and 69% in western Germany (20). However, in developing countries the prevalence of atopy has a considerable variability going from 21.5 % in Peru (17) and 26% in Estonia (18) to 89% in Costa Rica (21). In those children without current asthma symptoms, the prevalence of atopy also varies and the reported figures are 28.7% in Spain, 31.4% in Peru, 22% in Sweden, 9% in Estonia and 65.5% in Costa Rica (16-18, 21). This wide variability in the proportion of atopy observed in adolescents with current wheezing (ranging from 21.5% to 89%) and also in those without current wheezing (ranging from 9% to 65.5%), would support the idea that atopy may be just a parallel event in the pathogenesis of asthma (13). Furthermore, the overestimation of atopy as causative of asthma may have delayed the research on other important possible etiological mechanisms for the development of the disease (14), as those that eventually determined asthma symptoms in our non-atopic children and possibly in other individuals from underprivileged populations.

The similarly low proportion of atopy in children, with or without current asthma symptoms found in the present study is in agreement with other studies from Peru and Estonia, where current asthma symptoms were not related to atopy, decreased pulmonary function or BHR (17, 18). In contrast, a study done in Costa Rica (ISAAC phase II) found that atopy and maternal history of asthma were both associated with an increased risk for current wheezing, however, it is worth to mention that in the same study the proportion of atopy in children without current wheezing was 65.5% (21). Furthermore, the high prevalence of current asthma symptoms found in children from Costa Rica (24%) and Peru (26%) in which there was a remarkable difference in the prevalence of atopy (21% in Peru and 89% in Costa Rica) suggests that other factors unrelated with atopy (probably characteristic environmental factors present at each locality, different in nature and magnitude) are maybe more important as determinants of asthma symptoms or BHR and of the differences reported in the literature.

Socio-economic risk factors seem to be related with asthma symptoms even in developed countries. For instance, in a study from UK (15) the prevalence of wheezing was related to lower social class only in non-atopic individuals, but not in atopics. Recently, in Sweden, it has been reported that asthma, rhinitis and allergic sensitization is more common in lower than in higher socio-economic groups and this would be related with life style and environmental exposures (22). It is likely to surmise that environmental risk factors including those related to low social economic status may play an important role for the variability of symptoms, BHR and atopy in childhood.

The variability in the prevalence of BHR seems to be independent from the type of challenge methods employed, atopy, socioeconomic or development status and asthma symptoms. For example, the proportion of children without wheezing that had BHR to hypertonic saline found using the same ISAAC phase II protocol was 3% in Estonia, 30% in Sweden and 40% in Costa Rica (18, 21); the same significant variability in BHR

observed in children from different localities has also been described in adults (2). At present, the reasons for the reported variability in the prevalence of asthma symptoms, BHR and atopy are unknown. Although apparently not related with the prevalence of current asthma symptoms, atopy seems to be an important determinant in the variability of the reported BHR prevalence. In Swedish children BHR was present in 52% of atopic wheezing children and in 26% of those non-atopic who reported current wheezing whilst in Estonia, BHR was found in 30% of the atopic and in 6% of non-atopic wheezing children (18); in the present study the proportion was 83.3% and 62.3%, respectively. Maybe, more important than the difference found in the proportion of BHR between children with and without current wheezing (71.6 and 52.6%, respectively) is the magnitude of the difference (demonstrated by the fact that the PAR of BHR as a “cause” of current wheezing was no more than 40%). The latter suggests that other mechanisms independent from atopy and BHR would be also able to induce asthma symptoms in children.

Another finding of this study was the large proportion of children, with and without current wheezing, who were exposed to well-known airway irritant factors e.g. indoor kerosene and gas combustion, exposure to tobacco smoke, and probably to endotoxin, due to the high proportion of children living with stray dogs and cats as “pets”. In the neighborhood where the study was performed, the PM₁₀ exceeded 400 µg/m³ several times during autumn and winter. Environmental pollution exposure is an important and frequently present non-allergic factor capable to cause asthma symptoms and lung function decline in otherwise non-asthmatic children or to increase asthma symptoms severity (23-26). It is likely that the high proportion of children without current wheezing who had BHR to methacholine (52.6%) in our study could be, at least in part, explained by the long-standing exposure to mentioned indoor and outdoor atmospheric pollutants.

This study suggests that under special living conditions i.e.: burden of environmental harmful factors, mainly related to low socioeconomic status (smog, indoor pollution, infections), the prevalence of asthma symptoms and BHR to methacholine although high, remains independent from atopy. The latter is in agreement with recent publications suggesting that asthma is not simply the result of a polarized Th₂ response (13); other non-allergic mechanisms, maybe more important and present early in life would be involved as causative events (13), particularly those related to environmental conditions (27). It had been demonstrated that viral induced changes in cytokine responses can lead to recurrent wheezing and physician diagnosed asthma in infants (28, 29). In a birth-cohort study undertaken in the same city area where the present study was done, the prevalence of recurrent wheezing (3 or more episodes) during the first year of life was 40.3% and it was not related to personal or familiar atopy (30). Considering all the above given information, it is likely that the early respiratory exposure since birth to an “aggressive environment”, i.e. chemicals (smog and gases) and biomass combustion, biological (respiratory virus), as occurred with the children of this study, may result in modifications of innate inflammatory response early on life (29,31), which could result in the observed different relationship between bronchial responsiveness, atopy and symptomatic expression of disease. Although the long-term effects on the respiratory health of children who are continuously exposed to harmful environmental factors and having asymptomatic BHR are unknown, we speculate that the additive effect of both conditions might contribute to increase the population at risk for chronic obstructive pulmonary disease later in adulthood.

The higher prevalence of adolescent females with current wheezing in our study is concordant with previous studies using the ISAAC protocol and shown that after puberty

girls had more prevalence of current asthma after adjusted by confounders (32). The latter is consistent with our finding in ISAAC phase I in Santiago (unpublished data by the authors), where in girls the prevalence of current wheezing was higher than boys (64.6% vs. 35.4%, respectively). In the random sample of children 13-14 years old who participated in ISAAC Phase 1 in Santiago the proportion of females was 53.2%.

In conclusion, this study demonstrated that self-reported symptoms of current asthma correlated well with BHR to methacholine but not with atopy, suggesting that BHR and atopy may have different effects in wheezing symptoms. Furthermore, the similar proportion of atopy and high proportion of BHR found in those children without current asthmatic symptoms suggests a different relationship between symptoms, BHR and atopy in underprivileged populations exposed since birth to an aggressive environment.

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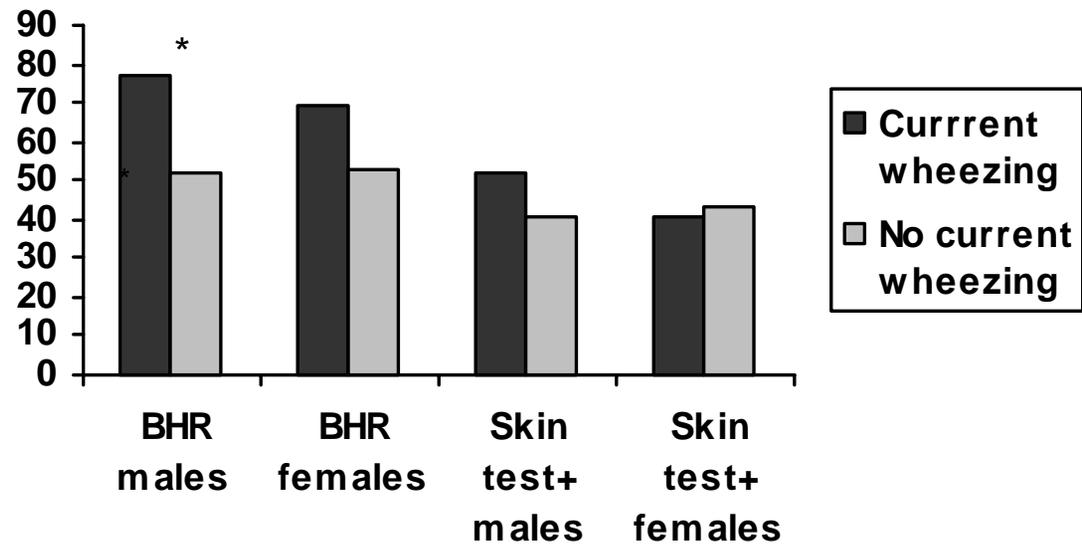
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Figure 1. Proportion of adolescents with and without current wheezing who had BHR to methacholine and positive skin prick test by gender.

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* p=0.028 comparing current wheezing vs. no current wheezing.