Outdoor pollen concentration is not associated with exercise-induced bronchoconstriction in children

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Abbreviated title: Pollen and exercise-induced bronchoconstriction.

Abbreviations list: AH = absolute humidity of air, CI = confidence interval, EIB = exercise-induced bronchoconstriction, FEV1= forced expiratory volume during 1 sec, grains per cubic metre = grains/m^3, grams per cubic metre = g/m^3, IgE = immunoglobulin E, IOS = impulse oscillometry, NO = nitric oxide, RH = relative humidity of air, R5 = impulse oscillometry resistance at 5 Hz, SD = standard deviation.

Abstract

Background: Free running exercise test outdoors is an important method to diagnose asthma in children. However, the extent of how much exposure to pollens of outdoor air affects the results of the test is not known.

Methods: We analyzed all reliable exercise challenge tests with impulse oscillometry in children (n=799) between January 2012 and December 2014 in Tampere University Hospital. Pollen concentrations at the time of the test were collected from the register of Biodiversity Unit of the University of Turku. We compared the frequency of exercise-induced bronchoconstriction and pollen concentrations.

Results: The analyses were restricted to birch and alder pollen as high counts of grass and mugwort pollen were so infrequent. The relative change in resistance at 5 Hz after exercise or the frequency of exercise-induced bronchoconstriction were not related to alder or birch pollen concentrations over 10 grains/m^3 (p=0.125-0.398). In logistic regression analysis comparing the effects of alder or birch pollen concentrations, IgE-mediated alder or birch allergy and absolute humidity over 10 g/m^3 only absolute humidity was independently associated with change in airway resistance (OR 0.32, CI 0.13-0.67, p-value 0.006).

Conclusions: In our large clinical sample, outdoor air pollen concentration was not associated with the probability of exercise-induced bronchoconstriction in free-running test in children while low absolute humidity was the best predictor of airway obstruction.

Introduction

Asthma is one of the most common chronic diseases among children and adults worldwide [1]. Asthma is characterized by reversible or variable bronchial obstruction associated with airway inflammation [2]. Exercise-induced bronchoconstriction (EIB) is a common finding in asthma and it can be provoked with exercise challenge testing [3]. Impulse oscillometry (IOS) is a fast and non-invasive lung function test requiring only minimal co-operation from the patient. IOS is therefore a commonly used and reliable lung function test in diagnosing asthma in preschool children [4,5]. It can be used to detect hyperresponsiveness in exercise or reversibility with inhaled beta-2-agonists [6,7]. Airway resistance at 5 Hz (R5) is the most reliable and repeatable parameter in diagnosing obstruction in children under school age [4].

There are different inflammatory mechanisms in asthma, of which the most common among children is allergic eosinophilic asthma often triggered by airborne allergens [8]. The allergen content of air varies a lot with climate. The key pollination period in Europe is about 6 months from spring to autumn [9]. Pollen from birch and other related trees, for example alder, are the major tree pollen type in Northern and Central Europe and causes the majority of pollen related symptoms [10].

Many previous studies have shown that there is an association between outdoor pollen counts and severity of nasal, conjunctival and bronchial symptoms in pollen sensitized patients [11–14]. Level of allergen exposure is also related to decreased quality of life [15]. There is evidence that pollen exposure is related to increased airway inflammation in asthmatic patients measured with exhaled nitric oxide (NO) or amounts of inflammatory cells [16–18]. In addition, increased pollen counts in outdoor air correlate with emergency room visits and asthma morbidity in several previous studies [19–23].

There are contradictory results on how short-term exposure to pollen affects lung function in asthmatics and if outdoor exercise test is affected by pollen count at the time of the test. To our knowledge, there is only one previous study in adults suggesting that pollen season affects the probability of EIB in outdoor exercise test in allergic subjects with asthma [24]. However, there are no previous studies in preschool children on the correlation between lung function measured with IOS and outdoor pollen count during exercise test. The aim of this study was to assess if pollen concentration of air affects the probability of EIB in outdoor exercise test with IOS in preschool children.

Materials and Methods

Study design and subjects

This study is a retrospective chart review, where all IOSs with exercise challenge test in children between January 2012 and December 2014 at Tampere university hospital, Finland, were analyzed. Children had been tested because of suspicion of asthma or need to assess efficacy of treatment in subjects with persistent asthma. We included only the first exercise test to the study if several tests had been performed in one child. Additionally, the recognized risk factors of asthma were collected from patient records, which were the results of allergy testing (skin prick test or allergen-specific Immunoglobulin E (IgE) -levels), atopy and atopic dermatitis, gestational age, birth weight and height, tobacco exposure during pregnancy and childhood, atopy and asthma in parents and siblings, pets at home, respiratory symptoms, number of episodes and hospitalizations of wheezing, other diseases, medications and whether asthma was diagnosed or not [25]. Contraindication for exercise challenge was significant respiratory infection within 4 weeks. The height adjusted to age as z-scores was also calculated [26]. Study was approved by the Ethics Committee of Tampere University Hospital (R15022).

Air properties and pollen concentration

The most important pollen particles in Finland are from birch (Betula), grass (in Finland the most common are timothy, meadow fescue and common meadow-grass) (Poaceae) and alder (Alnus). The allergens of alder and birch resemble each other structurally and immunochemically and cross-reactivity is common. In addition, we collected pollen data of mugwort (Artemisia vulgaris) and hazel (Corylus avellana). Pollen counts expressed as pollen grains per cubic metre (grains/m^3) of air at the time of each exercise test were collected from the register of Biodiversity Unit of the University of Turku, Finland. The Hirst-Burkard pollen trap, from where our data originated, was on the roof of the building next to the outdoor exercise test location [27].

Relative humidity (RH), temperature and pressure of air at the time of each exercise test were collected from the register of Meteorological Institute of Finland (license: CC BY 4.0, https://creativecommons.org/licenses/by/4.0/). Absolute humidity (AH) of air was calculated using RH, temperature and pressure. City of Tampere is located 61˚30' northern latitude and has a borderline humid continental climate/subarctic climate, daily mean temperature of -6.9 ˚C in February and +16.9 ˚C in July. AH is mostly under 5 grams per cubic metre (g/m^3) in winter and near 10 g/m^3 in summer [28].

IOS measurements and exercise challenge

IOS (Jaeger Gmbh, Würzburg, Germany) was measured by trained nurses according to international recommendations [29]. Possible technical problems during the measurements were noted, such as opening of the lips, postural problems, physical movement or holding of breath. To ensure the measurements meet the international quality criteria, technical properties of the measurements were reviewed retrospectively by trained physicians blinded to the outdoor air properties and pollen concentration [30,31]. The results were defined technically acceptable if they were repeatable and the coherence values were within acceptable limits. The exercise challenge was performed outdoors as free running. Exercise level was considered sufficient if heart rate (measured with FT4, Polar Ltd, Kempele, Finland) was > 85% of calculated maximal value (205-age/2) and the time of exercise was over 6 minutes [32]. Possible objective findings and symptoms (such as wheezing, cough and dyspnea) were documented. IOS was measured before exercise and 1-2, 5, 10 and 15 min after exercise. Children were given 300 µg of salbutamol (Ventoline evohaler via Babyhaler®) after these measurements and IOS was repeated 15-20 min after salbutamol inhalation. Reference values for IOS were chosen according to the age of the child [33]. R5 values were retrieved for analyses from each time point for each subject. The criterion for EIB was increase in IOS R5 ≥ 40% after exercise compared to baseline [6,29,31]. Criterion for significant bronchodilation effect was decrease in R5 ≥40% compared to baseline [7,29,34].

Statistics

Statistical analysis was made by using R-program version 4.0.2 (R foundation, Vienna, Austria). Alder and birch pollen concentrations were significantly skewed and thus they were categorized by using thresholds of 10 and 80 grains/m^3 (moderate and high pollen count, respectively) [11]. As cross-reactivity between alder and birch pollen is frequent and either of these pollen counts were over the threshold limit in only a small number of cases, they were combined in the analyses. Fisher exact test, Chi-square, Mann Whitney U-test or t-test were used in single parameter comparison between different groups. Logistic regression was used to compare the effects of AH, alder or birch pollen concentration over 10 grains/m³ and positive alder or birch IgE on frequency of EIB. Linear regression was used to compare the effect of AH, alder or birch pollen concentration over 10 grains/m³ and positive alder or birch IgE on R5. P-values under 0.05 were considered statistically significant.

Results

Demographic characteristics of the study subjects

In total 854 subjects had completed exercise test with IOS at study period and we collected data from those. After excluding technically unreliable IOS results (n=55) 799 (94%) children were selected for analyses. Ninety-seven percent of the study subjects were 3 to 7 years old (range between 3.0-14.1 years). The gestational age was under 37 weeks in 72 subjects (9.0%) of whom four subjects (0.5%) were born before 28 weeks. Birth weight was low (< 2500 g) in 50 subjects (6.3%).

EIB occurred in 13.9% of the study subjects after exercise challenge. Table 1 presents demographic characteristics of the study population (including only those with reliable IOS) and frequency of significant pollen concentrations at the time of exercise tests. Fifty-three percent of study subjects were atopic or had at least one IgE-mediated sensitization in prick or RAST testing and 35.8% had alder or birch IgE test positive (Table 1). Grass, mugwort and hazel pollen concentrations were over 10 grains/m^3 only in 3.0, 1.0 and 0.6% of tests and these pollen counts were excluded from analyzes due to lack of statistical power. Alder and birch pollen concentrations were over 10 grains/m^3 in 4.3 and 11.8% of the cases. Either alder or birch was over 10 grains/m^3 in 15.9% of cases.

Subject characteristics according to the presence of EIB are presented in Table 2. Children with EIB were on average slightly older and taller than those without EIB. Parental smoking and presence of asthma were not different between the groups. R5 before the test was lower, R5 rise after the test was higher and abnormal auscultation findings were more frequent in subjects with EIB. There were no differences in frequencies of birch or alder pollen counts being at least moderate (> 10 grains/m^3) or high (> 80 grains/ m^3) at the time of the exercise test between subjects with or without EIB.

Association between pollen concentrations and EIB

Table 3 presents subject characteristics according to either birch or alder pollen count at the time of test over or under 10 grains/m^3. Subjects who were tested when both pollen counts were low were on average slightly older and taller than those tested when either of the pollen counts were high. Interestingly, those tested when both pollen counts were low were slightly more often sensitized to birch or alder pollen. There was no statistically significant difference in the frequency of EIB between groups tested when either birch or alder pollen count was under or over 10 grains/m³ (14.0 % versus 13.4 %, p-value 0.968) (Figure 1). Also, there were no differences in baseline R5 (94.5 % versus 95.7 %, p-value 0.450) or mean change in R5 (17.4 % versus 15.5 %, p-value 0.449) (Table 3) between these groups. If the subjects were divided according to either alder or birch pollen being over or under 80 grains/m³ (720 vs 79 tests), or when either pollen was measurable (pollen grain count ≥ 1 grains/m³, 627 vs 127 tests), there were no significant differences between the groups in either frequency of EIB or change in R5 (p-value 0.125-0.613).

When we analyzed the subgroup of birch or alder pollen IgE positive subjects (n=286) there were no differences in frequency of EIB (16.7 % versus 21.4 %, p-value 0.711) or mean change in R5 (18.9 % versus 25.7 %, p-value 0.184) between subjects tested at higher and lower pollen concentrations (Table 4). In an additional subgroup analysis, when comparing tests with alder pollen concentration over or under 10 grains/m³, there were no differences in baseline R5 (95 % versus 96 %, p-value 0.702), frequency of EIB (13.6 % versus 20.6 %, p-value 0.368) or mean change in R5 (17.1 % versus 16.1 %, p-value 0.826) between subjects tested at higher and lower alder pollen concentrations. Similarly, when comparing tests with birch pollen concentration over or under 10 grains/m³, there were no differences in baseline R5 (95 % versus 96 %, p-value 0.436), frequency of EIB (14.3 % versus 10.6 %, p-value 0.417) or mean change in R5 (17.4 % versus 14.8 %, p-value 0.370) between subjects tested at higher and lower birch pollen concentrations.

The logistic regression analysis of EIB using alder or birch pollen over 10 grains/m^3, AH ≥ 10 g/m^3 and IgE positive birch or alder allergy showed that only AH ≥ 10 g/m^3 was correlated with EIB (OR 0.32, p-value 0.006) (Table 5). In multivariate linear regression analysis, using alder or birch pollen count over 10 grains/m^3, AH and positive birch or alder IgE-test, the R5 change after outdoor free running test was significantly related only to AH (regression coefficient -0.8769, p-value 0.003). When adding the asthma controller medication pause over 14 days, antihistamine medication or nasal corticosteroid as covariate, these did not significantly affect the results (p-value for all > 0.100) and only AH ≥ 10 g/m^3 correlated significantly with EIB incidence and change in R5 after the test. In multivariate regression analysis of any IgE-mediated sensitization, alder or birch pollen over 10 grains/m^3 and AH ≥ 10 g/m^3 both any IgE-mediated sensitization (p-value < 0.001) and AH ≥ 10 g/m^3 (p-value 0.003-0.006) were significantly correlated with change in R5 after outdoor free running test. If R5 as a percentage of reference values was modelled using the same cofactors as above, no factor remained statistically significant (p-values 0.346-0.390).

Discussion

To our knowledge, this is the first study concerning the connection between outdoor air pollen concentration and lung function tested with IOS. This study shows that the ambient outdoor pollen concentration does not significantly affect the incidence of EIB in children under school age undergoing free running test. There was no difference between allergic and non-allergic children.

In our previous study, we showed that AH of the outdoor air was an important physical factor affecting the probability of EIB in children, and higher AH was related to lower incidence of EIB [28]. As well in this study, the effect of AH on the incidence of EIB was higher than the effect of pollen concentration.

IgE-mediated allergy is thought to be a predisposing factor for asthma and high amounts of pollen in the air during exercise test has been thought to predispose for EIB. Several previous studies have shown that the high pollen concentrations are connected to allergic (rhinitis, conjunctivitis) and bronchial symptoms [13,14]. There is also evidence, that when allergen concentrations are high, the emergency department visits due to asthma and allergy increase [21–23]. The inflammation markers of asthma have been shown to increase in allergen exposure in several studies [17,18]. One small Finnish study [24] compared asthmatic men allergic and non-allergic to birch pollen in exercise test in winter season and in the spring pollen season. It was shown that there was more decrease in FEV1 after exercise in spring pollen season in allergic patients. Non-allergic patients had more decrease in FEV1 in winter season which was possibly due to different AH levels between seasons, but AH was not measured. However, studies have not managed to show that pollen concentrations have significant correlation with the change of lung function tests in all sorts of pollens [35–37].

In a Swedish study [35], grass but not birch pollen count was correlated to decreasing of lung function measured with spirometry during the pollen season. Effects on exercise test were not studied. The correlation was stronger in pollen sensitized children. In our study, high grass pollen counts occurred so rarely that reliable analysis could not be performed. Our data was originated from Hirst-Burkard-collectors located on the roof of the building next to the hospital. Grass pollen grains are heavy molecules and cause high concentrations only locally because they don't spread widely. However, there is no rich grass growth close to the place where the outdoor exercise test was performed, that could have caused local high pollen concentrations. In a Finnish study [38], grass pollen counts were compared between urban and rural areas in Helsinki metropolitan area. In the study, the grass pollen concentration increased with decreasing urbanity, but pollen concentrations can occasionally increase to such high levels that they can cause allergic symptoms also in most urban areas.

There are some limitations in our study. Because of the retrospective study design, all the clinical data of every subject was not in our reach. However, major confounders were covered. For example, the data about asthma or allergy medication and IgE-mediated sensitization were widely available in the patients. There are also minor confounders which could not be collected due to retrospective study design, such as asthma and allergy symptoms around the time of the exercise test and information about pollen concentrations in the subject´s living area before the exercise test. The climatic data was recorded every 10 minutes close to the study site, but pollen data was measured as daily concentrations. That may cause inaccuracy in pollen measurements in the exact time of exercise tests. Better assumptions about causality between pollen counts and EIB could be made with a longitudinal study design, but a longitudinal study of this extent would be difficult and expensive to conduct. There are several environmental and host-relating factors affecting EIB and the mechanism of EIB is complicated. Because of that, elimination of all confounding factors is impossible in practice. A priori power calculations were difficult since there was no previous definition for a clinically significant difference in the probability of EIB or the change in R5 to be used in a study like this. If 10% difference in EIB incidence or 10% change in R5 was considered clinically significant between low and moderate/high alder or birch pollen groups, our study had a statistical power higher than 80%. Due to lack of power, we could not study the effects of some allergens, most notably grass pollen.

In our study population, there are children whose asthma is diagnosed based on symptoms of repeatable obstructions and wheezing in very young age. Among these children, the prognosis of asthma is very good and spontaneous recovery is very common and allergies do not often develop. This may be a confounding factor in our study. Some of these children develop later allergies and allergic asthma, and because of our retrospective study design, we cannot analyse them as a separate subgroup. In clinical practice, there are patients who have bronchial symptoms in allergen exposure in pollen season but not in other seasons. If patients have difficult symptoms, they are treated during the pollen season based on symptoms and the exercise test is often scheduled outside allergy season. Due to elimination of the most severely symptomatic patients, there might be a selection bias in our study population. This study might be underpowered for studying the effects of markedly high pollen concentrations which patients can encounter in daily lives but not necessarily on the time of the exercise test.

In conclusion, in this large clinical real-life cohort, pollen concentrations did not significantly affect the results of outdoor exercise tests in children with suspected or diagnosed asthma. Our results suggest that there is no need to consider variations in pollen counts in mildly or moderately allergic children when interpreting clinical exercise test results or planning timing of the test. Further, our results demonstrate that absolute humidity is a more significant factor in exercise-induced bronchoconstriction than pollen count. In future studies, absolute humidity should be considered when studying the effects of pollen or other outdoor factors.

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Table 1. Subject characteristics and frequency of significant pollen concentrations at the date of exercise test in the study population with technically reliable IOS (n = 799). Data is either mean (SD) or percentages.

	Mean (SD) or %
Age (years)	5.4 (1.4)
Height (cm)	112.9 (9.6)
Males (%)	64.7
R5 change after exercise (%)	17.1 (26.1)
Exercise induced bronchoconstriction	13.9
Any IgE test positive	53.2
Alder or birch IgE test positive	35.8
Any asthma controller medication	56.4
Inhaled corticosteroids	54.9
Leukotriene receptor antagonists	11.1
Long-acting beta-agonists	7.0
Antihistamine	9.0
Nasal corticosteroid	2.3
Alder or birch pollen ≥ 10 grains/m^3 (%)	15.9
Birch pollen ≥ 10 grains/m^3 (%)	11.8
Alder pollen ≥ 10 grains/m^3 (%)	4.3
Grass pollen ≥ 10 grains/m^3 (%)	3.0
Mugwort pollen ≥ 10 grains/m^3 (%)	1.0
Hazel pollen ≥ 10 grains/m^3 (%)	0.6

Table 2. Subject characteristics, exercise test results and frequencies of moderate or high pollen grain counts in subjects with technically reliable IOS (n=799) divided into those with or without EIB. Values are either mean (SD) or percentages.

	EIB (-)	EIB (+)	
	n=688	n=111	p-value
Age (years)	5.4 (1.4)	5.7 (1.4)	0.009
Height (cm)	112.5 (9.5)	115.3 (9.6)	0.003
Iso BMI ($kg*m^{-2}$)	22.8 (4.7)	22.4 (4.5)	0.416
Gender Male (%)	64.0	69.4	0.317
Parental smoking (%)	28.5	34.2	0.262
Asthma in either parent (%)	34.4	36.9	0.687
Controller medication pause over 14 days (%)	96.5	97.1	0.974
Alder or Birch IgE test positive (%)	34.4	44.1	0.061
R5 of reference values (% of reference)	95.5 (17.3)	89.3 (15.3)	<0.001
R5 change after exercise (% change)	9.1 (12.8)	66.9 (31.6)	<0.001
Wheezing in auscultation (%)	6.5	51.4	<0.001
Alder or birch pollen ≥ 10 grains/m^3 (%)	16.0	15.3	0.968
Birch pollen ≥ 10 grains/m^3 (%)	12.2	9.0	0.417
Birch pollen ≥ 80 grains/m^3 (%)	9.4	5.4	0.227
Alder pollen ≥ 10 grains/m^3 (%)	3.9	6.3	0.368
Alder pollen ≥ 80 grains/m^3 (%)	0.7	2.7	0.156

Table 3. Subject characteristics and exercise test results in subjects with technically reliable IOS (n=799) divided according to alder or birch pollen count over or under 10 grains/m^3. Data is mean (SD) or percentage.

	< 10 grains/m^3	≥ 10 grains/m^3	
	n=672	n=127	p-value

Age (years)	5.5 (1.4)	5.0 (1.2)	0.001
Height (cm)	113.3 (9.6)	110.4 (9.0)	0.002
Gender Male (%)	65.3	61.4	0.457
Alder or Birch IgE test positive (%)	38.4	22.0	0.001
R5 of reference values (% of reference)	94.5 (17.4)	95.7 (15.9)	0.450
Obstruction before exercise (%)	3.3	2.4	0.792
R5 change after exercise (% change)	17.4 (26.3)	15.5 (24.8)	0.449
Exercise induced bronchoconstriction (%)	14.0	13.4	0.968
Marked symptoms in exercise (%)	10.9	11.8	0.874
Wheezing in auscultation (%)	12.8	12.6	1.000

Table 4. Subject characteristics and exercise test results in subjects with technically reliable IOS and positive birch or alder IgE test (n=286) divided according to alder or birch pollen over or under 10 grains/m³. Data is mean (SD) or percentage.

	< 10 grains/m³	≥ 10 grains/m³	
	n=258	n=28	p-value
R5 of reference values (% of reference)	93.8 (17.3)	92.0 (15.9)	0.608
Obstruction before exercise (%)	3.5	3.6	1.000
R5 change after exercise (% change)	18.9 (25.5)	25.7 (27.1)	0.184
Exercise induced bronchoconstriction (%)	16.7	21.4	0.711

Table 5. Incidence of EIB explained with absolute humidity ≥over 10 g/m^3, alder or birch pollen count over 10 grains/m^3 and alder or birch IgE positive using logistic regression (n = 799).

	OR	95% CI			p-value
Alder or birch pollen ≥ 10 pcs/m^3	0.90	0.43	–	1.73	0.754
AH ≥ 10 g/m^3	0.32	0.13	–	0.67	0.006
Alder or birch IgE positive	1.46	0.94	–	2.27	0.093
Alder or birch pollen ≥ 10 pcs/m^3 * Alder or birch IgE positive	1.78	0.51	–	5.83	0.346