Conference Paper

ALLERGIC DISEASES IN RELATIONSHIP WITH ENVIRONMENTAL FACTORS IN A POPULATION OF SCHOOL CHILDREN IN ZAGREB, CROATIA

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Most scientists believe that increasing number of people with allergic diseases may be connected with some aspects of the "Western lifestyle". This paper discusses data obtained from questionnaires originally designed by the International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee concerning exposure to different environmental factors. The study included 1047 children.
Allergic and non-allergic groups showed statistically significant differences in the attendance of kindergarten, vaccination against pertussis, pertussis infection, and parasite infestation. Exposure to tobacco smoke during pregnancy and exposure to dampness and moulds also entailed a risk for allergy. We speculate that changing some conditions, such as use of carpets and use of feather pillows, were connected with the expression of allergic diseases. Some correlations were consistent with earlier observations of other authors, while others differed and need further confirmation on a larger sample.

KEY WORDS: *allergies, carpeting, childhood, damp, ISAAC, moulds, parasites, pertussis, tobacco smoke*

Epidemiological studies are trying to find the answers to the questions about the influence of environmental factors on allergic diseases and asthma. Differences in the prevalence of allergic diseases between countries with different lifestyles are of special interest.

To date, there have been no comprehensive epidemiological studies which would investigate the causative relationships between environmental factors and allergic diseases in Croatia. Our previous study (1) investigated the prevalence of symptoms of allergic diseases in school children in Zagreb. The 12-month prevalences of wheezing, allergic rhinoconjunctivitis, and atopic eczema were 6.0 %, 12.1 %, and 7.8 %, respectively.

The aim of this paper is to discuss other data obtained from questionnaires originally designed by the International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee concerning exposure to different environmental factors.

SUBJECTS AND METHODS

Sampling strategy

Eighteen schools were randomly chosen out of 102 elementary schools in the city of Zagreb, Croatia. The study included all children aged 10 years 0 months to 10 years 11 months (4th grade). A total number of 1364 children was considered sufficient for the calculations of rates.

Questionnaires

We used questionnaires (2, 3) developed by the original ISAAC collaborators. All modules were

translated from English into Croatian by physicians specialised in asthma and allergy, according to translation guidelines (4). The questionnaires were then distributed, completed by the subjects' parents and returned. A total of 1047 questionnaires were returned and analysed. Data from supplementary modules about past and present living and exposure conditions will be discussed below.

Data management and analysis

The responses to the questionnaires were first checked for inconsistencies, which were eliminated in a phone conversation with parents. Data were then entered into the computer and analysed. Statistical analyses were performed using SPSS software (SPSS for Windows 10.0, SPSS Inc., Chicago, IL, USA). The differences between investigated groups were calculated with the chi-square test. A probability value of $p<0.05$ (two-tailed) indicated a statistically significant difference.

RESULTS

Participants

All selected schools participated in the study. The response rate of parents averaged 77 % (range 59-94 %). 47.4 % of subjects were boys and 52.6 % were girls. 88.6 % of subjects were born in Croatia, 9.3 % in Bosnia and Herzegovina, while the rest were born in other countries. 36.2 % of subjects had symptoms of allergic disease (wheezing and/or allergic rhinoconjunctivitis and/or atopic eczema) at any time in the past. The symptoms of allergic disease were present in 39.9 % of boys, and 32.9 % of girls. The higher prevalence of allergic symptoms in boys compared with girls was statistically significant ($p=0.021$).

Exposure to microorganisms

Table 1 shows the details of exposure to microorganisms. 81.2 % of children went to kindergarten between 3 and 6 years of age. 85.0 % of children who had allergy symptoms and 79.0 % of children who did not have allergy symptoms went to kindergarten. The difference between the compared groups was statistically significant ($p=0.023$).

99.0 % of all subjects had been vaccinated against pertussis according to the vaccination calendar; Pertussis vaccine was received by 97.9 % of subjects with and 99.7 % of subjects without allergy symptoms. Again, the difference between the two groups was statistically significant ($p=0.010$).

Table 1 *Exposure to microorganisms*

	Kinder-garten		Pertussis vaccine		Pertussis infection		Parasite infestation	
	n	%	n	%	n	%	n	%
Total n=1047	850	81.2	1037	99.0	41	3.9	43	4.1
Allergic n=379	322	85.0[x]	371	97.9[x]	28	7.4[x]	24	6.3[x]
Non-allergic n=668	528	79.0[x]	666	99.7[x]	13	2.0[x]	19	2.8[x]

Each item is expressed as absolute and relative value. The relative value is calculated for the total number of included children (n=1047), for the total number of children who had allergy symptoms at any time in the past (n=379), and for non-allergic children (n=668)
[x]Statistically significant differences

3.9 % of subjects had pertussis earlier in their lives. Pertussis was acquired by 7.4 % of subjects with and 2.0 % without allergy symptoms, with a statistically significant difference between the groups ($p<0.001$).

4.1 % of subjects had parasite infestations earlier in their lives. Parasite infestation was acquired by 6.3 % of subjects with and 2.8 % without allergy symptoms, with a statistically significant difference between the groups ($p=0.010$).

Exposure to other environmental factors

Table 2 shows data on exposure to other environmental factors. Mothers of 20.4 % of children smoked during pregnancy; Mother's smoking during pregnancy was present in 24.3 % of subjects with and 18.2 % without allergy symptoms. The difference between the groups was statistically significant ($p=0.023$).

Damp spots on the walls or ceilings were found in the homes of 7.1 % of subjects at present; Damp spots were present in the homes of 9.8 % of subjects with and 5.5 % without allergy symptoms, and the difference between the groups was statistically significant ($p=0.015$).

8.6 % of subjects had damp spots on the walls or ceilings of their homes during their first year of life. Damp spots were present in the homes of 11.9 % of subjects with and 6.7 % without allergy symptoms,

Table 2 *Exposure to other environmental factors*

	Mother's smoking during pregnancy		Damp spots at present		Damp spots during the child's first year of life		Molds or fungus at present		Molds or fungus during the child's first year of life		Loose carpets at present		Bare floor at present		Feather pillow at present	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Total n=1047	213	20.4	74	7.1	90	8.6	50	4.8	64	6.1	621	59.3	193	18.4	507	48.4
Allergic n=379	92	24.3[x]	37	9.8[x]	45	11.9[x]	28	7.4[x]	34	9.0[x]	199	52.5[x]	94	24.8[x]	136	35.9[x]
Non-allergic n=668	121	18.2[x]	37	5.5[x]	45	6.7[x]	22	3.3[x]	30	4.5[x]	422	63.2[x]	99	14.8[x]	371	55.5[x]

Each item is expressed as absolute and relative value. The relative value is calculated for the total number of included children (n=1047), for the total number of children who had allergy symptoms at any time in the past (n=379), and for non-allergic children (n=668)
[x]Statistically significant differences

with a statistically significant difference between the groups (p=0.006).

Moulds or fungi on the walls or ceilings were found in the homes of 4.8 % of subjects at present. Moulds or fungi were present in the homes of 7.4 % of subjects with and 3.3 % of subjects without allergy symptoms, with a statistically significant difference between the groups (p=0.005).

6.1 % of subjects had visible moulds or fungi on the walls or ceilings in their homes during their first year of life. Moulds or fungi were present in the homes of 9.0 % of subjects with and 4.5 % without allergy symptoms, with a statistically significant difference between the groups (p=0.006).

20.4 % of subjects had fitted carpets, 59.3 % had loose carpets, and 18.4 % had a bare floor in their bedrooms at present. 52.5 % of subjects with and 63.2 % without allergy symptoms had loose carpets. 24.8 % of subjects with and 14.8 % without allergy symptoms had a bare floor. Bedroom floor covering shows a statistically significant difference between allergic and non-allergic groups (p<0.001).

At present 15.2 % of subjects use foam pillows, 24.5 % use synthetic fibre pillows, and 48.4 % use feather pillows. 2.3 % of subjects do not use a pillow. 35.9 % of subjects with and 55.5 % of subjects without allergy symptoms use feather pillows. The type of the pillow used was connected with a statistically significant difference between allergic and non-allergic groups (p<0.001).

We also analysed whether a child shared the bedroom with other people, whether any pets were kept inside the child's home, whether a child had contacts with animals outside his or her home, which fuel was used for cooking and heating, whether a child's home was air conditioned, what kind of windows were there in a child's bedroom, and what kind of bedding was used. All items were analysed for the present and for the first year of a child's life. There were no statistically significant differences between allergic and non-allergic groups for these items.

DISCUSSION

High participation in the study has made possible some initial considerations about the influence of environmental factors on the onset of allergic diseases. However, in some cases the sample size is not sufficient for final conclusions.

The prevalence of allergy symptoms (asthma, and/or allergic rhinoconjunctivitis, and/or atopic eczema) was greater in boys. This was also the case with asthma symptoms, and allergic rhinoconjunctivitis symptoms considered elsewhere. In contrast, we did not find any difference in the prevalence of atopic eczema between the sexes (1).

Exposure to different microorganisms in early childhood is considered to be an important factor in the development of allergies. Hygiene hypothesis postulates that higher living standards and improved personal hygiene lead to an increased risk of allergic sensitization (5). Conversely, frequent viral and bacterial infections in early childhood might prevent allergic diseases (5, 6). As a biological basis for this hypothesis, it is suggested that a lower frequency of infections in childhood, due to a reduced opportunity for contacts, may influence the mechanisms of dysregulation of the immune response. Immunologic

studies have also suggested a down-regulation of IgE production by viral and bacterial infections (7, 8).

Children who attend a child care facility, nursery school, or a kindergarten, and children in large families, who have older siblings, have an increased opportunity for cross-infections, and according to the hygiene hypothesis, would be at lower risk for the development of allergy (9, 10). Our results showed that there was no statistically significant difference between allergic and non-allergic children who went to a child care facility or nursery school. Among the children who went to kindergarten at the age of 3-6 years, there was a significantly higher proportion of allergic than non-allergic children, which is the opposite to what the hygiene hypothesis suggests. A possible explanation is that the majority of children were nursed at home during their first year of life, and started to go to a facility during their second and third year. They could develop allergic disease before increased risk of infections in the facility. We have not found statistically significant differences between allergic and non-allergic children regarding their siblings, which might be explained by a small proportion of large families in our community.

There is increasing evidence that the relationship between infections and the development of allergic diseases depends on the child's age when the infections occur and on the type of causative pathogen (11, 12). Infections which occur before the age of 2 years may affect subsequent sensitisation to allergens and emergence of asthma, with two quite opposite effects. Particularly important are respiratory syncytial viral infections, which are strongly associated with the onset of asthma and allergic sensitisation later in life. Other viral and bacterial infections might be protective against allergies. After the age of 2 years the most important pathogen is rhinovirus, which is mostly associated with exacerbations among children who already have asthma. Pathophysiologic mechanisms are not clear.

Several studies suggested that vaccination is less protective against allergic diseases than natural infection with viral or bacterial pathogens. A study of villagers from Guinea-Bissau, West Africa, after a measles epidemic showed that villagers who had measles infections had approximately one-third the rate of allergen sensitisation compared with those who had been vaccinated (13). Another study of Japanese schoolchildren with complete records of tuberculin skin test results after BCG vaccination showed that children who exhibited strong positive tuberculin skin tests (natural exposure) had fewer symptoms of asthma, rhinitis, and eczema, lower levels of serum IgE and Th2 cytokines, and higher levels of Th1 cytokines than children with weak positive tuberculin skin tests (vaccination) (14).

Our results about vaccination against measles and tuberculosis showed that all participants received BCG vaccine, and only two participants did not received the measles vaccine. This rendered impossible the comparison between allergic and non-allergic groups. Similarly, none of the participants had tuberculosis, and a comparison between groups could not be done. Only 25 participants had measles, without statistically significant differences between allergic and non-allergic groups.

Interesting results were obtained for pertussis. Among children who received pertussis vaccine there was a significantly lower proportion of allergic than non-allergic children, and among children who had pertussis infection the proportion of allergic children was significantly higher. This contradicts the results obtained in the aforementioned studies, and suggests that pertussis vaccine might have a protective effect against the development of allergic diseases, and that pertussis infection might contribute to their onset. However, there is a possibility of bias, because only a small proportion of subjects have been exposed, and these results have to be assessed on a larger sample.

Several studies suggested that parasitic infestations protect against allergy, presumably by blocking the production of allergen-specific IgE, while others showed that certain helminth infestations, particularly ascaris, can provoke atopic responses (15). It seems that communities in rural Africa where worm infections are common, extensive and frequently life-long have a very low prevalence of asthma and immediate type hypersensitivity to inhalant allergens (16, 17). In studies from China and Brazil, current ascaris infection was a strong, independent risk factor for allergic skin test reactivity and wheezing (15, 18).

Our results regarding parasitic infestations showed a pattern similar to pertussis. There was a significantly higher proportion of allergic children among participants who had worm infestations. Parasites should be considered possible triggers for the later onset of allergic diseases. However, only a small proportion of subjects were exposed, and again, a larger sample is needed for the confirmation of our findings.

Tobacco smoke has been identified as the main source of indoor air pollution, and up to 70 % of children are exposed to it in their home during their early years of life (19, 20). Numerous studies have shown that parental smoking increases symptoms and the frequency of asthma attacks in asthmatic children (21, 22), and may also increase the risk of childhood asthma (23). Maternal smoking during pregnancy and early childhood is associated with impaired lung growth and diminished lung function (24, 25). Children of smoking mothers are also more prone to develop respiratory tract infections than children of nonsmoking families (19, 26).

The effect of secondary smoke exposure on the development of atopy in children is still a matter of debate. While some studies found a correlation between atopic sensitization and environmental tobacco exposure (27, 28), others failed to show such a correlation (29, 30). Our results showed no difference between allergic and non-allergic children regarding current tobacco smoke exposure. On the contrary, children of mothers who smoked during pregnancy and their early childhood were at higher risk of allergy symptoms. There was a significantly higher proportion of allergic children among the participants whose mothers smoked during pregnancy. Among participants whose mothers smoked during the first year of their life, the proportion of allergic children was also higher, although the difference was not statistically significant.

Another important environmental factor is exposure to different indoor allergens - house dust mites, animal dander, cockroaches, moulds, and pollens (11). Allergen exposure over the first few years of life probably induces sensitisation. Continued exposure can maintain inflammation in the nose and lungs, and its interaction with other factors can lead to the onset of an allergic disease.

House dust mites and different types of moulds and fungi grow better in warm places with humidity over 45 % (31, 32). Our results showed that living in damp places and places with visible moulds or fungi entails a risk for allergy. The proportion of allergic versus non-allergic children was significantly higher among participants who had lived in such places. There were far more allergic than non-allergic children among those who had lived in damp places in their first year of life, and also among participants who were exposed to such conditions at the time of the study.

Facts about indoor conditions showed statistically significant differences between allergic and non-allergic subjects regarding bedroom floor covering and the type of pillow they use. Interestingly, there was a smaller proportion of allergic children among those who had loose carpets in their bedrooms and a greater proportion of allergic children among those who had bare floors. The proportion of allergic children was also smaller among participants who used a feather pillow. These results oppose expectations. However, the results for the first year of childrens' lives were similar, but showed no statistically significant difference between allergic and non-allergic subjects. We believe that parents changed their environment once they suspected or were told their child had or could develop an allergic disease.

CONCLUSION

Our earlier results (1) showed a relatively low prevalence of allergic diseases and asthma in a school population in Zagreb, Croatia. In this paper we discussed the relationship between different environmental factors and allergic diseases and asthma in the same population. We found some correlations, mostly consistent with observations of other authors, which include parasitic infestations, exposure to tobacco smoke and damp environment with moulds or fungi. However, other correlations showed a different pattern. The most interesting finding is a potential protective effect of pertussis vaccine against the development of allergic diseases. Greater proportions of allergic children among those who went to kindergarten and those who have a bare floor in their bedroom, and lower proportions of allergic children among those who have loose carpets in their bedrooms and those who use feather pillow are other findings that need further confirmation on a larger sample. Therefore, we have recently increased our sample size to more than 3000 subjects and started ISAAC Phase Two which includes objective measurements of environmental exposure to airborne allergens.

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Sažetak

UTJECAJ ČIMBENIKA OKOLIŠA NA POJAVU ALERGIJSKIH BOLESTI U POPULACIJI ŠKOLSKE DJECE U ZAGREBU

Većina istraživača smatra da je sve veći broj ljudi s alergijskim bolestima povezan s pojedinim aspektima "zapadnjačkog načina života". Cilj je ovog članka razmotriti podatke dobivene iz originalnih upitnika koje je razradio ISAAC-ov tim stručnjaka, a koji se odnose na izloženost različitim čimbenicima iz okoliša.
U istraživanje je uključeno ukupno 1047-ero djece u dobi od 10 godina 0 mjeseci do 10 godina 11 mjeseci iz zagrebačkih osnovnih škola.
Prirodna izloženost različitim mikroorganizmima, vakcinacije te izloženost drugim čimbenicima okoliša, kao što su duhanski dim, vlaga, plijesni itd. analizirani su kao potencijalni rizični čimbenici za pojavu alergijske bolesti.
U skupini ispitanika koji su išli u vrtić između treće i šeste godine života bio je statistički značajno viši postotak djece sa simptomima alergijske bolesti. Među ispitanicima koji su primili pertusisnu vakcinu opažen je značajno niži postotak alergične djece, dok je kod djece koja su preboljela pertusis taj postotak bio značajno viši. Također je dobiven značajno viši postotak alergične djece među ispitanicima koji su preboljeli infestacije crijevnim parazitima.
Djeca majki koje su pušile za vrijeme trudnoće i tijekom prve godine života djeteta, bila su pod većim rizikom za pojavu alergijske bolesti, za razliku od djece koja su duhanskom dimu izložena u sadašnjosti. Ekspozicija vlazi i plijesnima predstavlja rizik za alergijsku bolest. Među ispitanicima koji imaju tepih u spavaćoj sobi, kao i kod onih koji rabe pernati jastuk, bio je značajno niži postotak alergične djece, dok je kod djece koja imaju goli pod taj postotak bio značajno viši.
Neki naši rezultati u skladu su s prethodnim opažanjima drugih autora, dok se neki razlikuju. Najzanimljiviji rezultat je potencijalni zaštitni učinak pertusisne vakcine protiv razvoja alergijskih bolesti. Ove će rezultate svakako biti potrebno provjeriti na većem uzorku.

KLJUČNE RIJEČI: *hripavac, izloženost mikroorganizmima, paraziti, pljesni, pušenje, simptomi alergije, uporaba tepiha, uvjeti stanovanja, vlaga*

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