

Concentration of IgE in children during ragweed pollination season

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Abstract Genetic and environmental factors are responsible for running allergic diseases. The aim of this study was to compare the values of total- (t-IgE) and allergen-specific IgE (s-IgE) to *Ambrosia artemisiifolia* L. (*Amb a*) in children with sensitization to *Amb a* during ragweed pollination season, who experienced seasonal symptoms of allergic rhinitis (rhinorrhea, post-nasal drip, nasal congestion, itching, sneezing) and asthma (coughing, especially at night, wheezing, shortness of breath, chest tightness). Ragweed pollen grains were collected in Virovitica (rural area) and Zagreb (urban area)—cities with the same geographical width and elevation—during ragweed pollination seasons (July–October in 2006 and 2007), and their count was estimated. Concentration of t-IgE and s-IgE in pollination season was determined in

serum of children with symptoms of allergic diseases. The total count of ragweed pollen grains (PG) differed significantly between Virovitica and Zagreb in both years, 2006. and 2007. In Virovitica it was significantly greater than in Zagreb. There was no statistically significant seasonal difference in both, t-IgE and s-IgE, respectively. No correlation was found between pollen grain count and the concentration of IgE's. To clarify the induction of IgE synthesis in children with sensitization to *Amb a*, further studies are needed.

Keywords Aerobiology · Allergy · IgE · Pollen grain count · Ragweed

1 Introduction

Ragweed (*Ambrosia artemisiifolia* L., *Ambrosia elatior* L.), an annual wind-pollinated, seasonal potent allergenic weed, has spread all over the Europe, from north to south, particularly in the central and eastern European countries: France (Fumanal et al. 2007), Italy (Zanon et al. 2002), Switzerland (Bohren et al. 2005), Austria (Vogl et al. 2008), Poland (Stach et al. 2007), Czech Republic (Rybniček et al. 2000), Hungary (Makra et al. 2005). A few studies have recently presented the seasonal distribution of *Ambrosia artemisiifolia* L. in Croatia (Peternel et al. 2005; Štefanić et al. 2005; Cvitanović et al. 2007).

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Children with ragweed allergy have increased serum concentration of both, total (t-IgE) and allergen (*Ambrosia artemisiifolia*)-specific (s-IgE), respectively. Synthesis of IgE depends on genetic factors and exposure to a broad array of environmental allergens (Nickel et al. 2002). Human (Baldacci et al. 2001) and animal studies (Nelde et al. 2001) have indicated that the concentration of IgE could correlate with the degree of immune stimulation, i.e., frequency, dose and route of allergen exposure (Nelde et al. 2001).

The aim of this cross-sectional and comparative study was to compare the values of t-IgE and s-IgE to *Ambrosia artemisiifolia* L., (*Amb a*), in children with verified sensitization to the ragweed pollen allergen during the season of ragweed pollination. *Amb a* comprises the major allergens of *Ambrosia artemisiifolia*, ragweed grown in Croatia (Peternel et al. 2006). It was hypothesized that the greatest sensitization of ragweed-sensitized children originates from the area with higher concentration of ragweed pollen grains.

2 Materials and methods

The study was conducted in Virovitica (low-sized city, i.e., about 23,000 inhabitants, agricultural surroundings) and Zagreb (big-sized city, i.e., about 800,000 inhabitants, industrial surroundings). Virovitica is a town on the northern slopes of Bilogora Mountain in the region of Podravina (45°50'N; 17°23'E; elevation 122 m), near Hungary. Economy is based on farming, forestry, wood and timber, food manufacturing etc. Zagreb, the capital of Croatia, is situated in south-western part of the Panonian Basin, between Medvednica Mountain and the banks of the Sava river (45°49'N; 16°02'E; elevation 120 m). Economy is based on metal-processing, production of electrical appliances, pharmaceutical and chemical industry etc. Pollen grains were collected and determined simultaneously in both cities from July 15 to October 20. The study was performed during the pollination seasons in 2006 and 2007.

2.1 Pollen grain counting analysis

Pollen samples were collected simultaneously in both cities, by standardized volumetric method (Peternel

et al. 2005) using Hirst pollen and spore traps (Burkard Manufacturing Co. Ltd., UK in Virovitica and Lanzoni, Italy in Zagreb) during the period from July 15 to October 20. Daily counts were made using a light microscope, and the result was expressed as the average of pollen grains per cubic meter (PG/m³) of air.

2.2 Patients

The study included 204 children aged 2–16 years (median = 8 years), with verified mono-sensitization (positive skin prick test and increased specific IgE) to the ragweed pollen allergen (*Ambrosia artemisiifolia* L.). The samples of 161 (20/100,000 inhabitants) consecutive outpatients referred by primary care physicians to Srebrnjak Children's Hospital in Zagreb (2006 *N* = 78; 2007 *N* = 83) and 43 (185/100,000 inhabitants) consecutive outpatients referred by primary care physicians to General Hospital, Virovitica (2006 *N* = 16; 2007 *N* = 27), who experienced seasonal symptoms of allergic rhinitis (rhinorrhea, post-nasal drip, nasal congestion, itching, sneezing) and asthma (coughing, especially at night, wheezing, shortness of breath, chest tightness) underwent a complete diagnostic evaluation, including total and allergen-specific IgE determination. Asthma was diagnosed based on GINA guidelines (Global Strategy for Asthma Management and Prevention 2006). Allergic rhinitis was diagnosed according to the Clinical Practice Guidelines (Bousquet et al. 2003).

2.3 Serum IgE determination

The concentration of total IgE was determined by Microparticle Enzyme Immunoassay (MEIA) method and reagents (Abbott, USA) with the use of mouse monoclonal anti-IgE antibodies, and measurement of fluorescence on an IMx autoanalyzer of the same manufacturer. The method sensitivity is 0.048 kIU/l (data from package insertion).

The concentration of allergen specific IgE to *Amb a* was measured by UniCAP method (Phadia AB, Uppsala, Sweden) where high sensitivity is achieved by the use of a three-dimensional cellulose carrier (Paganelli et al. 1998), on a selective UniCAP 100 autoanalyzer (Phadia, Uppsala, Sweden). The confidence interval is from 0.35 to 100 kIU_A/l. Calibrators for determination of total and specific IgE were

calibrated according to the World Health Organization Second International Reference Preparation for Human IgE (WHO 2nd IRP 75/502).

2.4 Statistics

Data were stored and prepared for statistical analysis using the Microsoft Office Excel 2000 software (Microsoft, USA). MedCalc (Medisoftware Mariakerke, Belgium) was used for data processing (MedCalc 2006). The variables with asymmetric distribution were presented by median (M) and interquartile rank (IQR), and Wilcoxon test was used for comparison of these variables. To examine the differences with categorical variables, chi-square test was used (Zar 1984). The values $P < 0.05$ were considered statistically significant.

3 Results

3.1 Pollen

It was observed that the total count of ragweed pollen grains (PG) differed significantly between Virovitica and Zagreb in seasons 2006 and 2007 (Fig. 1). The daily count of PG (median, interquartile range—IQR) was significantly greater in Virovitica than in Zagreb (Table 1) in both seasons (in 2006: $P = 0.0015$, and in 2007: $P < 0.0001$, respectively). The difference between the number of days with pollen grains exceeding 20 PG/m^3 was also statistically significant ($P = 0.001$).

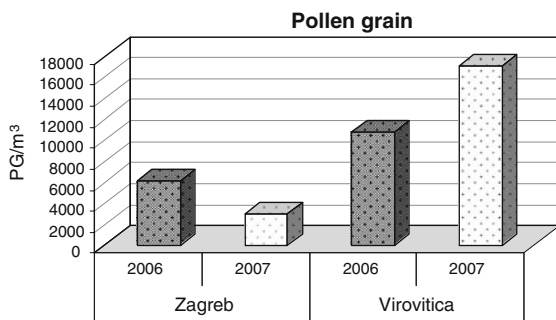


Fig. 1 Comparison of ragweed pollen grain count in Zagreb and Virovitica during the pollination season in 2006 and 2007 (from July 15 to October 20)

3.2 IgE concentration

Although there was no statistically significant seasonal difference ($P > 0.05$) in t-IgE concentrations neither between Zagreb and Virovitica nor between the years 2006 and 2007, IQR values in Virovitica tend to be higher in 2007 (176.6–627.9 kU/l) than in 2006 (183.0–325.7 kU/l), Table 1. The IQR values of specific IgE concentration to *Amb a* (Table 2) tend to be higher in Zagreb (in both 2006 and 2007 season: 2.53–62.55 kU_A/l and 2.51–65.10 kU_A/l) than in Virovitica (in both 2006 and 2007 season: 1.42–19.18 kU_A/l and 1.97–44.70 kU_A/l). However, there was no statistically significant seasonal difference ($P > 0.05$) in specific IgE concentrations between selected groups of children.

A good correlation between the concentration of t-IgE and s-IgE (Table 3) was supported by the coefficient of rank correlation values, pointing to a significant correlation between the concentration of t-IgE and s-IgE. No correlation was found between pollen grain count and the concentration of IgE (total and specific IgE).

4 Discussion and conclusion

The results of the present study showed the ragweed grain number to be higher in the city with agricultural surroundings (Virovitica) than the ragweed grain number in the city with industrial surroundings (Zagreb) during the same period of ragweed pollination. Nevertheless, there was no statistically significant difference between concentrations of total and specific IgE to *Amb a*, in children from both cities. Hypothesis, that the greatest sensitization of ragweed sensitized children originates from the area with higher concentration of ragweed pollen grains, was not confirmed.

The present study was performed on children from the northern part of Croatia with a moderate continental climate. Close neighbourhood of Hungary, i.e., the most heavily affected country with ragweed (Makra et al. 2004) and different industrial and agricultural surroundings are possible reason for a higher pollen grain count per cubic meter of air in Virovitica than in Zagreb. Šikoparija et al. has already shown higher values of ragweed pollen index in the rural (Ruma) than in urban (Novi Sad) areas

Table 1 Comparative values of ragweed pollen grain count in Zagreb and Virovitica during the pollination season in 2006 and 2007 (from July 15 to October 20)

	Zagreb		Virovitica	
	2006	2007	2006	2007
Total number (m ³)	6104	3012	10781	17036
Daily range (m ³)	0–619	0–275	1–779	1–1683
Daily median (m ³)	33	24	42	82
Daily IQR (m ³)	2–119	3–84	19–246	17–451
Days with pollen grains/m ³ >20 (N)	36	34	53	54

IQR interquartile range

Table 2 Concentration of total and specific IgE in children from Zagreb and Virovitica during pollination season in 2006 and 2007

	Zagreb		Virovitica	
	2006 <i>N</i> = 78	2007 <i>N</i> = 83	2006 <i>N</i> = 16	2007 <i>N</i> = 27
Total IgE (kU/l)				
Range	23.0–1904.8	22.7–2915.0	100.0–1828.0	100.0–1892.0
Median	240.2	239.4	199.0	332.5
IQR	114.8–451.6	112.6–482.1	183.0–325.7	176.6–627.9
Specific IgE (kU _A /l)				
Range	0.39–>100.0	0.39–> 100.0	0.64–> 100.0	0.42–> 100.0
Median	18.95	18.40	2.96	13.20
IQR	2.53–62.55	2.51–65.10	1.42–19.18	1.97–44.70

Table 3 Spearman's coefficient of rank correlation (*r*) between total and specific IgE in children from Zagreb and Virovitica during seasonal ragweed pollination in 2006 and 2007

Total IgE:specific IgE	Zagreb		Virovitica	
	2006	2007	2006	2007
<i>r</i>	0.608	0.655	0.685	0.785
<i>P</i>	<0.0001	<0.0001	0.0080	0.0002
95% CI	0.455–0.727	0.517–0.753	0.287–0.882	0.558–0.903

95% CI 95% confidence interval

(Šikoparija et al. 2006) of the southern Panonnian Valley (i.e. southern–eastern Panonnian Valley). The occurrence of pollen allergy is influenced by genetic predisposition to allergy, and environmental factors, i.e., allergen exposure (O'Meara et al. 2005), local vegetation (Cariñanos et al. 2002), rainfall and relative humidity, wind direction and speed (Breton et al. 2006; Makra et al. 2004). Relatively greater rate of children in Virovitica (185/100,000 inhabitants) than in Zagreb (20/100,000 inhabitants) with sensitization to *Amb a* experiences seasonal symptoms of rhinitis and/or asthma during the ragweed pollination period. In paediatric population the prevalence of allergic rhinitis and pollinosis in Croatia is showing

continuous rise (Kavurić-Hafner and Matika Šetić 1996), but there is a significant difference between coastal and continental areas, being moderate in the coastal (Cvitanović et al. 2007) and low in continental region (Prus and Culjak 2004; Stipić-Marković et al. 2008). According to Prus and Culjak the increased number of subjects hypersensitive to ragweed allergen is in correlation with the increase of air pollen grain count per cubic meter of air (Prus and Culjak 2004).

Amb a 1, an acidic 38-kDa nonglycosylated protein, is the most important ragweed allergen. Accordingly, about 95% of ragweed-sensitive subjects have increased serum concentration of IgE to

Amb a 1 (Wopfner et al. 2005). According to a 14-year study in Austria (Jäger 2000), the higher the ragweed pollen grain count, the higher the percentage of subjects with increased specific IgE, especially those with respiratory allergy. An epidemiological study in Croatia has shown that rural residence and smoking can be predictors of increased total serum IgE for women (Macan et al. 2007). The present study was performed on children from the northern part of Croatia with a moderate continental climate. The concentration of total IgE in children from Virovitica tends to be higher in 2007 than in 2006. In adults from coastal region of Croatia, specific IgE to *Amb a* ranged from 16 to >100 kU_A/l, median 45 kU_A/l (Cvitanović et al. 2007). Our results for children from continental regions have shown that specific IgE to *Amb a* ranged from 2.96 kU_A/l (children from Virovitica in 2006) to 18.95 kU_A/l (children from Zagreb in 2006). Specific IgE tends to be higher in children from Zagreb than in children from Virovitica. The analysis of the concentrations of total IgE and specific IgE to *Amb a* has revealed the patients with higher mean concentration of total IgE to also have an increased concentration of specific IgE to *Amb a*.

Although very low concentrations of ragweed pollen grains (i.e., about 10 PG/m³ of air) are sufficient to trigger allergic reactions in sensitive subjects (Taramarcaz 2006), the allergy symptom score is significantly higher when ragweed pollen grain count is on peak days (Cvitanović et al. 2007). Although the pollen grain count was found to be higher in rural environment, rural residence is not the sole predictor of higher IgE concentration. Hygiene hypothesis (i.e., that natural microbial exposures may have preventive effects against the development of allergy) must also be taken into consideration.

We are aware that this study has some limitations, such as a relatively small number of children, especially in rural area, no clinical data, no air pollution data, and no meteorological data.

This preliminary study demonstrated that concentrations of ragweed pollen grains in both cities were sufficient to induce high IgE synthesis in sensitive children. To clarify the induction of IgE synthesis in children with sensitization to *Amb a*, future studies should address other biomarkers involved in these processes.

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