ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2007, 14, 87-91

INCIDENCE OF BETULACEAE POLLEN AND POLLINOSIS IN ZAGREB, CROATIA, 2002-2005

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Peternel R, Milanović SM, Hrga I, Mileta T, Čulig J: Incidence of Betulaceae pollen and pollinosis in Zagreb, Croatia, 2002-2005. *Ann Agric Environ Med* 2007, **14**, 87-91.

Abstract: Pollen allergy is characterized by seasonal allergic manifestations affecting patients during the plant pollen season. The aim of this study was to analyze the Betulaceae pollen pattern in Zagreb (2002-2005) and to determine the incidence of sensitization to these pollen types in patients with seasonal respiratory allergy. Twenty-four-hour pollen counts were carried out using volumetric procedure. Skin prick test were performed on a total of 864 patients aged 18-80< in Zagreb between 2 January – 31 December 2004. Pollen of the representatives of the family Betulaceae accounted for a significant proportion of total pollen (34% on an average), predominated by *Betula* pollen and considerably lower proportion of *Alnus* sp. and *Corylus* sp. pollen. Alder and hazel pollen first occurred in the air in February throughout the study period. The highest airborne pollen concentration of these taxa was recorded in February and March. The birch pollen season generally peaked in April. Only 2.67% of patients showed birch pollen monosensitization. The proportion of patients with polysensitization to Betulaceae pollen was considerably greater (12.88%), whereas polysensitization to Betulaceae, Poaceae and *Ambrosia* pollen was recorded in the highest proportion of patients (26.23%). According to age, the highest and lowest rate of allergy was recorded in the 31-50 and >51 age groups, respectively (46.22% vs 23.12%). Female predominance was observed across all age groups. The patients with monosensitization to birch pollen had the most severe symptoms in April. In the patients with poylsensitization to alder, hazel and birch pollen who developed cross-reaction, initial symptoms occurred as early as February, with abrupt exacerbation in March and April. The most severe condition was observed in the patients allergic to birch, hazel, alder, grass and ragweed pollen, with symptoms present throughout the year and exacerbation in spring and late summer months.

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Key words: aerobiology, Betulaceae, pollen grains, pollen allergy, Zagreb (Croatia).

INTRODUCTION

Pollen allergy (pollinosis) is a common disease caused by hypersensitivity reaction of the respiratory tract and eye conjunctivae to pollen grains. It refers to seasonal allergic manifestations affecting patients during the plant pollen season. Seasonal allergy is characterized by the recurrence of symptoms. It is well established that inhalation of pollen grains induces respiratory allergy symptoms in sensitized individuals. These are clinically manifested as rhinitis, rhinoconjunctivitis and bronchial asthma [9]. In northern, central and eastern Europe, early springtime allergic airway diseases are commonly caused by pollen from the family *Betula*ceae [19, 25, 28]. This contains the genera *Alnus* (alder), *Betula* (birch) and *Corylus* (hazel) [5]. *Alnus* and *Corylus* are the first airborne pollen types of the season [24]. The high frequency of sensitization might be caused by the apparent high degree of cross-reactivity with

Received: 30 March 2006
Accepted: 10 January 2007

the *Betula* pollen allergen [4, 6, 27]. Establishing a dose-response relationship between pollen exposure and symptoms is difficult and highly individual. The range of severity for individuals is quite broad, and symptoms often reflect concurrent exposure to several allergens. Response usually increases with ongoing short-term exposure (priming), and exposure involves aerosol fractions besides intact pollen grains. For allergic patients who are particularly sensitive to tree pollen grains, it appears that the onset of clinical manifestations depends on the total amount of pollen disseminated [11]. However, it has been pointed out that birch pollen concentrations greater than 30 grains in m^3 per 24 h trigger severe symptoms, and values greater than 80 grains in m^3 per 24 h produce allergic symptomatology in 90% of patients [3]. The hazel pollen gives allergic reaction at concentrations of 20-30 grains in m^3 per 24 h and alder pollen at concentrations of 50 grains in m^3 per 24 h [23, 26]. Prevention of symptoms is not easy to achieve, given that amounts of pollen vary from year to year, apparently with pollination cycles that are species specific and liable to differ from region to region [11].

The aim of this study was to analyze the *Betula*ceae pollen pattern in Zagreb (2002-2005) and to determine the incidence of sensitization to these pollen types in patients with seasonal respiratory allergy.

MATERIAL AND METHODS

The study was performed in the city of Zagreb in the 2002-2005 seasons. Zagreb is situated in the central part of Croatia with a continental climate. A 7-day VPPS 2000 Hirst volumetric spore trap (Lanzoni, Bologna, Italy) was used for pollen sampling. The sampler was placed at a height of 19.7 m on the roof of the Grič Observatory in the centre of the City of Zagreb (45° 49' N and 15° 59' E, 157 m above the sea level). The sampler absorbs 10 L air per minute, allowing for determination of pollen concentration at 2-hour intervals. It is supplied with a timer which moves adhesive tape (2 mm/h) for pollen grains to stick to. The tape was removed twice weekly, cut to a length corresponding to 24-hour pollen sampling. Samples were examined under a light microscope, magnification ×400, to determine pollen type and count per 1 m^3 air per 24 h.

In this study, a total of 864 patients aged 18-80< initially reporting to the Jordanovac University Hospital for Lung Diseases in Zagreb and Zagreb Polyclinic for Lung Diseases between 2 January – 31 December 2004, were examined (history and skin-prick test). Skin prick test was performed by the usual method. Allergen preparations of grass pollen – *Alopecurus pratensis*, *Agrostis alba*, *Anthoxantum odoratum*, *Cynodon dactylon*, *Dactylis glomerata*, *Festuca elatior*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Secale cereale*, *Triticum sativa*, *Zea mays*; weed pollen – *Ambrosia elatior*, *Artemisia vulgaris*, *Artemisia absinthium*, *Solidago canadensis*, *Rumex acetosella*, *Plantago lanceolata*, *Parietaria judaica*, *Parietaria officinalis*, *Taraxacum officinale*, *Urtica dioica*, *Humulus lupulus*, *Cannabis sativa*; and tree pollen – *Corylus avellana*, *Betula verrucosa*, *Sambucus nigra*, *Tilia cordata*, *Pinus nigra*, *Robinia pseudoacacia*, *Salix alba*, *Populus alba*, *Platanus acerifolia*, *Alnus incana*, *Quercus robur*, *Aesculus hippocastanum* (Institute of Immunology, Zagreb) were dialyzed and the extracts dissolved in a solvent, a mixture of 50% glycerol solution in buffered saline with the addition of a stabilizer. A solution of histamine HCl at a concentration of 1 mg/ml (5.43 mmol/l) was used as positive control, and 50% glycerol solution in phosphate buffer as negative control. The mean values of positive reaction had urtica diameter of 5-10 mm, and erythema diameter of 10-30 mm.

RESULTS

During the 4-year period (2002-2005), pollen of the representatives of the family Betulaceae accounted for a significant proportion of total pollen (34% on average), predominated by *Betula* pollen (21%) and a considerably lower proportion of *Alnus* sp. and *Corylus* sp. pollen (6% and 7%, respectively) (Fig. 1). Total annual airborne pollen concentrations of the Betulaceae family varied from year to year, showing a regular annual alternating pattern of high and low concentrations (29,437 pg m^{-3} in 2002, 9,029 pg m^{-3} in 2003, 17,715 pg m^{-3} in 2004, and 9,834 pg m^{-3} in 2005). The same applied to pollen of particular family representatives (*Alnus*, *Corylus*, *Betula*). Birch was found to disseminate the highest pollen amounts, whereas alder and hazel pollen was present in comparable but considerably lower amounts, with the exception of hazel pollen in 2002 when its concentration almost reached that of birch pollen (Fig. 2). Alder and hazel pollen first occurred in the air in February throughout the study period, except for 2003 when the beginning of the pollen season was delayed by a month. The highest airborne pollen concentration of these taxa was recorded in February and March. The birch pollen season generally peaked in April, except for 2002, when it occurred in March. Accordingly, pollen of the family Betulaceae was present in the air from February untill

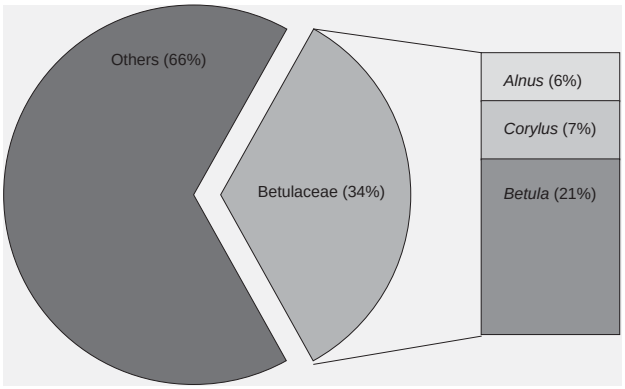

Figure 1. Mean four-year (2002-2005) airborne pollen concentration of the family Betulaceae and its representatives (*Alnus*, *Corylus* and *Betula*) in City of Zagreb.

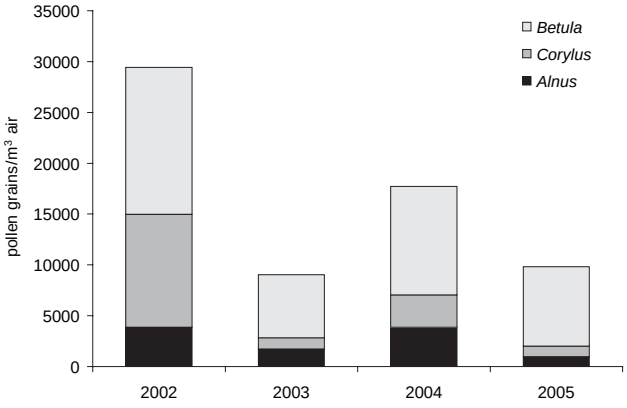

Figure 2. Annual sums of airborne Betulaceae pollen in Zagreb 2002-2005.

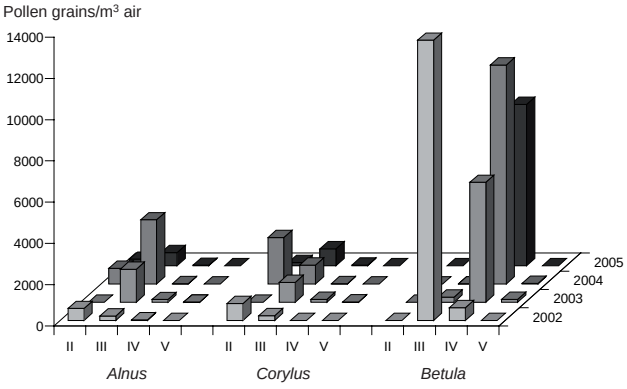

Figure 3. Monthly variation of airborne Betulaceae pollen in Zagreb 2002-2005.

April throughout the study period (Fig. 3). The number of days with the airborne pollen concentration exceeding the level provoking the symptoms of allergic reaction ranged from 7-20, i.e. 16-31% for alder, depending on the duration of the pollen season. For hazel, the percentage ranged from 18% in 2003 and 2005 through 43% in 2002, whereas birch showed a very high percentage in 2002 (83%) and 2003 (93%), with a decline in 2004 (52%) and 2005 (53%)

Table 1. Number of days with airborne pollen concentration exceeding the threshold value Vt for allergic reactions to *Alnus*, *Corylus* and *Betula* (2002-2005), and length of pollen season (in days).

Year	*Alnus*	*Corylus*	*Betula*
	V_t > 50 p.m.$^{-3}$ air	V_t > 20 p.m.$^{-3}$ air	V_t > 30 p.m.$^{-3}$ air
2002	16/61	21/48	39/47
2003	9/34	9/51	31/33
2004	20/63	19/62	16/31
2005	7/43	10/55	16/30

(Tab. 1). Out of 864 study patients, 678 (78.47%) patients showed pollen allergy (225 to birch pollen in combination with other pollen types), whereas allergy to other inhalation allergens (mite, animal hair, feather, epithelium, and *Alternaria* and *Cladosporium* spores) was recorded in 21.5% of study patients. Only 2.67% of patients showed birch pollen monosensitization. The proportion of patients with polysensitization to *Betula*ceae pollen was considerably greater (12.88%), whereas polysensitization to *Betula*ceae, Poaceae and *Ambrosia* pollen was recorded in the highest proportion of patients (26.23%). According to age, the highest and lowest rate of allergy was recorded in the 31-50 and >51 age groups, respectively (46.22% vs 23.12%). Female predominance was observed across all age groups, lowest in the 18-30 age group (53.63%) and highest in the >51 age group (67.31%). According to type and combination of pollen species, allergic women predominated in all groups (*Betula*; Betulaceae; *Betula*, Poaceae; Betulaceae, Fagaceae; *Betula*ceae, *Ambrosia* and Betulaceae, Poaceae, *Ambrosia*). Male predominance was found for allergy to the following pollen groups: *Betula*, *Ambrosia*; Betulaceae, Poaceae i *Betula*, Poaceae, and *Ambrosia* (Tab. 2, Fig. 4).

Following the airborne pollen distribution described above, almost all study patients suffered the highest symptom severity in March and April. The patients with monosensitization to birch pollen alone had the most severe symptoms in April. In the patients with poylsensitization

Table 2. Age and sex distribution of respiratory allergy patients according to different pollen type.

Sensitized to pollen	Patients (Skin Prick Test Positive) age							
	18-30		31-50		51<		Total	
	♂	♀	♂	♀	♂	♀	♂	♀
Betula	0	2	2	1	0	1	2	4
Betulaceae	1	4	5	13	2	4	8	21
Betula, Poaceae	1	3	0	1	1	0	2	4
Betula, *Ambrosia*	1	0	2	1	2	2	5	3
Betulaceae, Fagaceae	0	2	4	7	0	8	4	17
Betulaceae, Poaceae	3	4	7	2	3	5	13	11
Betula, Poaceae, *Ambrosia*	6	5	3	2	1	1	10	8
Betulaceae, *Ambrosia*	5	6	12	17	6	8	23	31
Betulaceae, Poaceae, *Ambrosia*	15	11	12	13	2	6	29	30
Total	32	37	47	57	17	35	96	129
% (age)	30.66		46.22		23.12			

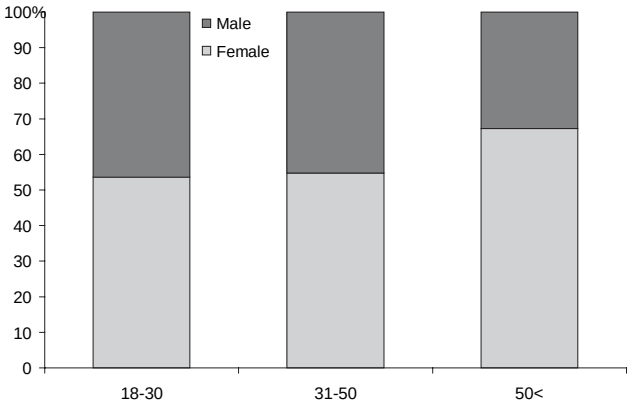

Figure 4. Sex distribution of patients with positive skin-prick test within age groups.

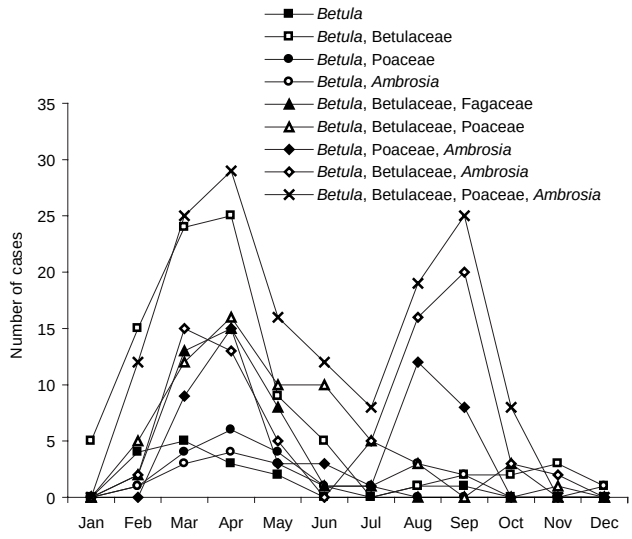

Figure 5. Annual pattern of allergic symptomatology in monosensitized and polysensitized patients.

to alder, hazel and birch pollen who developed cross-reaction, initial symptoms occurred as early as February, with abrupt exacerbation in March and April. The most severe condition was observed in patients allergic to birch, hazel, alder, grass and ragweed pollen, with symptoms present throughout the year and exacerbation in spring and late summer months (Fig. 5).

DISCUSSION

Pollen components of the atmosphere do not present the same allergenic significance for humans. The occurrence of pollen allergy is influenced by local vegetation, length of allergen exposure and genetic predisposition to respiratory allergy [10, 17]. It is well documented that inhalation of specific species of pollen grains causes clinical symptoms of respiratory allergy [16]. The concentrations of airborne Betulaceae pollen in the atmosphere of Zagreb vary for both annual total amount of pollen and annual values of *Betula*, *Alnus* and *Corylus* over the 4 years of recording. A wide range of factors affect the production, dispersion and transportation of airborne pollen (wind direction and speed, rainfall and relative humidity during the flowering season [21, 28]. Many studies suggest that it is necessary to record and correlate the airborne pollen concentrations with the most important meteorologic parameter values [14, 18, 28]. However, in spite of the great annual variation in pollen concentration, a certain regular pattern has been observed in the pollen concentration ratio of particular *Betula*ceae family representatives and in the periods of particular pollen shedding during the 4-year study. Thus, airborne birch pollen predominated in all study years, regularly following alder and hazel pollen. This phenomenon along with cross-reactivity between the representatives of Betulaceae family as well as between the pollen of Betulaceae family pollen and of some other plants, accounts for the prolonged seasons of symptoms in many patients [13]. It was also observed in our study that patients who developed symptoms during the flowering season of the plants to the pollen of which they were allergic. In the predominant group of patients polysensitized to the Betulaceae, Poaceae and *Ambrosia* pollen (26.23%), the symptoms persisted from March to October. Our results are consistent with those reported from many studies conducted worldwide [2, 7, 8, 9].

Our study showed that pollen was the major sensitizing aeroallergen in patients with symptoms of respiratory allergy. The rate of 78.47% of patients with pollen allergy recorded in our study was only slightly lower than the rate of such patients reported in an epidemiological study carried out in Iran [12]. In most European and North American countries, the allergens with highest sensitization rates among patients with nasal symptoms are also pollen, followed by house dust mites and cat dander [22]. *Betula* pollen poses a considerable clinical problem for up to 10%-20% of the population in northern and central Europe [26]. This rate greatly exceeds the rate of only 2.67% recorded in our study. The fivefold rate of allergy to *Betula*ceae pollen (12.88%) was still below that reported from Spain (18.52%), where alder pollen was predominant in the family *Betula*ceae [15]. The sex distribution of our patients (a slight predominance of allergic women in all age groups) was consistent with the results reported from the Basque Country [1], Spain and Portugal [20] but differed from reports from Greece and Iran [9, 12]. Age is also an important variable. Most studies have focused on students or military recruits. We included patients belonging to 3 age groups (18-30; 31-50 and 51< years). In our population sample, the highest rate of pollinosis was found in the 31-50 age group (46.22%). In the study conducted in the Basque Country that included the population aged 10-40, the highest rate of allergy was found in the 20-30 age group (12.5%) [1].

In conclusion, pollen is the major aeroallergen provoking seasonal respiratory allergies in Zagreb, with a high proportion of patients allergic to pollen of the family Betulaceae representatives. Our results are compatible with

similar studies carried out in many countries. This study was based on 4-year investigations; additional studies over a longer period are needed to provide a more profound insight into the relationship between pollen content in the air and seasonal allergic manifestations affecting patients during plant pollen seasons.

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