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CORRELATION BETWEEN CONJUNCTIVAL PROVOCATION TEST RESULTS AND CONJUNCTIVAL SYMPTOMS IN POLLINOSIS - PRELIMINARY REPORT

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Abstract: The aim of the study was to investigate the correlation between the level of reactivity in conjunctival provocation test and conjunctival symptoms that develop during the pollination season in grass allergic patients. Conjunctival provocation test with grass pollen allergens was performed in 22 patients suffering from pollinosis. During grass pollination season all patients monitored their symptoms with Symptoms Score Cards. A parallel measurement of the level of grass pollen count was carried out on a daily basis by volumetric method. The mean grass pollen count which triggered the reaction in individual patients depended on the results of conjunctival provocation test. The lowest pollen count level was observed in cases of patients with positive conjunctival provocation test at low allergen extract concentration of 160 and 500 BU/ml, whereas the highest count in cases of 1,600 BU/ml. The difference between the results was found to be statistically significant. A threshold grass pollen concentration for conjunctival symptoms was established at the level of 22 grains/m^3. We conclude that the patients with pollinosis and high reactivity in conjunctival provocation test develop conjunctival symptoms earlier during grass pollination season than the patients who are characterised by lower reactivity during conjunctival provocation test.

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INTRODUCTION

Pollinosis occurs in 10–20% of European inhabitants [10]. Multicentre trials investigating the prevalence of allergic diseases in Poland showed that 8.7% of adults and 8.9% of children suffer from seasonal rhinitis and rhinoconjunctivitis [12]. In the Polish population,patients suffering from pollinosis are most frequently allergic to wild grasses and rye pollens [9]. Pollinosis is a multi-organ disease - allergic reaction is usually apparent in nasal mucous membrane and in conjunctiva; however the lower respiratory tract may be also affected. Type I immunological reaction, dependent on the presence of specific IgE antibodies, mast cells and eosinophil cells, forms the basis of this disease [1, 10, 20]. Itching is the typical symptom of allergic conjunctivitis. Moreover, redness of the eyes, lacrimation and mucous discharge in the conjunctival sac may also appear in the course of this disease.

Meticulous history taking combined with positive results of skin prick tests enable establishing of the correct diagnosis in the majority of patients with

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pollinosis [1, 9]. Confirmation of the presence of specific serum IgE antibodies by the means of immunodetection method may also contribute to the diagnostic process. Provocation tests with allergens are auxiliary diagnostic tools in identification of the allergic disorders [10]. During the conjunctival provocation test (CPT), allergens are administered in the conjunctiva and thereafter the symptoms caused by the organism's reaction are evaluated. Specific provocation test, which mimics natural exposure to allergens, enables confirmation of the clinical relevance of sensitization in doubtful cases. Furthermore, CPT may represent a model for the study of cells and mediators involved in the pathophysiology of allergic inflammation [3]. Valuable information has been gained about the ocular allergic response thanks to CPT [6]. Additionally, many studies have confirmed the value of conjunctival provocation test with the use of allergens [5, 6, 7, 11].

MATERIAL AND METHODS

22 patients aged 19–52 years, mean 27,5 ± 7,8 years, were included in this study. Male subjects constituted over 2/3 of the group. All patients were diagnosed as suffering from intermittent (seasonal) allergic rhinoconjunctivitis due to grass pollen allergens. In 8 subjects, allergy to grass pollens evoked symptoms of asthma coexisting with rhinoconjunctivitis. In all study participants, the IgE-dependant character of the disease was confirmed (CAP $\geq$ 2). In all patients positive skin prick test weal for grass allergens was at least as large as histamine control reaction. None of the patients had clinically relevant sensitization to late-pollinating trees or perennial allergens. Furthermore, none of the patients underwent specific immunotherapy with grass pollen allergens.

Conjunctival provocation test (CPT) with grass pollen allergen extract was performed in all 22 patients, according to a standardised procedure. A lyophilised pollen allergen extract of 6 grass species (*Holcus lanatus*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*, *Festuca elatior*) together with diluent was supplied by Allergopharma J. Ganzer KG, Reinbeck, Germany. One drop of increasing concentrations of the allergen extract was administered every 10 minutes in the interior conjunctival sac of each eye alternately. The initial concentration of allergen used for the procedure was 5 BU/ml, the highest, 5,000 BU/ml. The titration was performed in half logarithmic steps (factor 3.2); subsequent allergen concentrations used in the procedure were: 50 BU/ml, 160 BU/ml, 500 BU/ml and 1,600 BU/ml. Conjunctival provocation test was performed at least one month prior to the grass pollination season. The test result was regarded as positive when more than a half of the conjunctiva was injected (redness) and the eye was itching or showed general irritation. A course of conjunctival reaction to the subsequent allergen concentrations was recorded in detail.

All patients kept Symptom Score Cards for the first 3 weeks during the grass pollen season, which enabled quantitative analysis of the conjunctival symptoms of pollinosis. Aerobiological measurements carried out for many years showed that the grass pollination season in Poland takes place at the end of May and last throughout June and July [15, 16]. The patient self-observation period covered the early phase of grass pollen dissemination season. Every day, patients assessed the presence of ocular manifestations (redness, itching and lacrimation) using recommended scale from 0–3, where 0 meant lack of symptoms, 1 - mild symptoms with minimal inconvenience, 2 - moderate, and 3 - severe symptoms, that interfered with everyday activity and/or sleep. Only rescue medications (oral or topical antihistaminics used only when required) were permitted during the time of observation.

The course of grass dissemination season was evaluated with the use of aerobiological measurements, based on constant analysis of grass pollen count in the air using continuous volumetric method (Seven-Day-Recording Volumetric Spore Trap - Burkard Manufacturing Co. Ltd., UK).

RESULTS

Analysis of the results of conjunctival provocation test with grass pollen allergens. In all 22 patients, CPT gave positive results, with no subjects reacting at low level of allergen concentration (5 or 50 BU/ml). Five patients developed positive CPT at medium concentration of 160 or 500 BU/ml (nearly every fourth patient), whereas half of the group showed reaction at the concentration of 1,600 BU/ml. Slightly more than one quarter of the patients reacted the level of 5,000 BU/ml (Tab. 1).

In every fourth patient, itching and eye irritation was the first reaction to the allergen provocation. However, in the majority of subjects (3/4 patients) both symptoms (itching and redness) appeared simultaneously. Itching was observed in every third patient at the allergen concentration of 160 or 500 BU/ml, and in the following 13 patients at the level of 1,600 BU/ml. Only every eleventh patient experienced itching at the level of 5,000 BU/ml (2 subjects). Redness coexisted with itching in every fourth patient at the allergen concentration of 160 or 500 BU/ml in half of the patients at the level of 1,600 BU/ml, and in over one third of subjects at the allergen concentration of 5,000 BU/ml (Tab. 2).

Analysis of conjunctival symptoms during grass pollens dissemination season. In the analysed early phase of grass pollens' dissemination season (20 May–5 June 2003) in Warsaw, the mean daily grass pollen count was 9–143 grains per 1 m^3 of the air (mean 51.5 ± 32.3). During the aforementioned period, one subject did not experience any conjunctival symptoms (however, nasal manifestations were present). Itching/eyes irritation developed

Table 1. Positive result of conjunctival provocation test in correlation with the concentration of grass pollen allergen extract administered to the conjunctivae.

Concentration of grass pollen allergen extract (BU/ml)	No. of patients with positive CPT	Fraction
160	1	0.05
500	4	0.18
1.600	11	0.50
5.000	6	0.27
Total	22	1.00

Table 2. Sequence of conjunctival symptoms appearance after grass pollen allergen provocation.

Initial conjunctival symptom	No. of patients	Fraction
Itching	6	0.27
Redness	-	-
Both itching and redness	15	0.73
Total	22	1.00

Table 3. Sequence of conjunctival symptoms appearance during the grass pollen dissemination season.

Initial conjunctival symptom	No. of patients	Fraction
No symptoms	1	0.04
Itching	11	0.50
Redness	5	0.23
Both itching and redness	5	0.23
Total	22	1.00

Table 4. Conjunctival Symptom Score Sum (with regard to symptoms which appeared at the threshold level of grass pollen count).

Symptom Score Sum.	No. of patients	Fraction
0	8	0.37
1	8	0.36
2	3	0.12
3	1	0.05
4	1	0.05
6	1	0.05
Total	22	1.00

Table 5. Correlation between daily mean grass pollen count evoking conjunctival allergic reaction and the level of reactivity during conjunctival provocation test.

Concentration of grass pollen allergen extract evoking positive CPT (BU/ml)	Grass pollen count			
	min.	max.	mean	Standard Deviation
160	22	22	22.0	0.0
500	19	35	24.5	7.1
1,600	19	118	66.5	37.6
5,000	11	94	47.0	30.2
Total	11	118	51.5	32.1

as the first symptom among ocular manifestations in half of the patients, and redness in a quarter of the subjects. One quarter of the patients developed both symptoms at the same time (Tab. 3).

Grass pollen count at which one quarter of patients with pollinosis showed allergic reaction was regarded as the threshold level during the early phase of grass pollen dissemination season. In our study, this was found to equal 22 grains per 1 m³ of the air for the appearance of conjunctival symptoms. During the day, when the threshold level was reached, every third patient did not experience conjunctival symptoms, whereas in more than one third of subjects symptom score equalled 1. Conjunctival symptom score was 2 or more [2, 3, 4, 6] in 6 (fraction 0.27) (Tab. 4).

Analysis of the correlation between the results of conjunctival provocation test and ocular manifestations during the grass pollen dissemination season. No statistically significant correlation was found between the sequence of symptoms appearance during conjunctival provocation test with the grass pollen allergen and during the grass pollen dissemination season. $chi^2 = 2.647$; $p>0.05$

The mean daily pollen count which triggered reaction to allergen (conjunctival symptom score of minimum 2) was 52 pollen grains per 1 m³ of the air. There was a significant scatter because the standard deviation was 32 grains per 1 m³ (Tab. 5). The mean grass pollen count which triggered the reaction in individual patients depended on the results of conjunctival provocation test. The lowest pollen count level was observed in cases of patients with positive conjunctival provocation test at the levels of 160 and 500 BU/ml, whereas the highest count (66.5 grains/m³) in cases of 1,600 BU/ml. However, in cases of positive results of the provocation test observed at the level of 5,000 BU/ml, the reaction during the season appeared when the mean grass pollen count reached the value of approximately 47. The difference between the results was found to be statistically significant. In cases of positive provocation test results appearing at the level of 1,600 BU/ml, the grass pollen count values evoking conjunctival reaction during the season were significantly higher than in cases when the positive CPT result was apparent at an allergen extract concentration level of 500 BU/ml ($F = 2.423$; $p < 0.05$).

DISCUSSION

Numerous reports confirm the diagnostic value of CPT, which is an acceptable alternative to nasal provocation test in patients with allergic rhinitis, even if they have no conjunctival symptoms. Riechelman *et al.* reported that nasal provocation test and CPT in house dust mites allergy yielded concomitant results in 90% of the subjects successfully tested [18]. In their study, Bertel *et al.* conclude that the results they obtained with CPT confirm its high antigenic quality. They find CPT as a particularly

useful, rapid, and safe clinical test [2, 19]. In their opinion, it is the only test able to establish a relationship between ocular manifestations and specific IgE. In our study, CPT was performed one month prior to the grass pollen dissemination season. Such timing was chosen because of the seasonal variation of the CPT described in different studies. Moller *et al.* reported that the priming effect during the pollination season (they studied birch pollen allergy) is retained for about a year [13]. The method used in our study to evaluate CPT is widely accepted. However, attempts have been made to overcome the subjective evaluation of the CPT by objective measurements of the vascular reaction with digital analysis [8].

The two most obvious symptoms of allergic conjunctivitis are itching and redness, the former being reported in up to 80% of patients. In the study of Rimas *et al.*, itching appeared before erythema in 83% of subjects during conjunctival provocation test [19]. In contrast, only in a quarter of our study group itching appeared before erythema during conjunctival provocation test, whereas in the majority of cases (3/4 of the group) both symptoms occurred simultaneously.

Reports dealing with the correlation between the clinical sensitivity, the onset of clinical symptoms and the pollen airborne content are scarce. Negrini *et al.* tried to determine in their study a threshold concentration of *Parietaria* pollen, i.e. a minimum amount of *Parietaria* pollen required do provoke allergic symptoms [14]. In that study, mild symptoms, probably due to the most sensitive part of the population, were correlated with levels of 15 Parietaria pollen grains/m³. In other studies, the threshold value was found to be 25 pollen grains for grasses [4]. The threshold value for conjunctival manifestations in our group of patients was 22 grass pollen grains/m^3. In a very similar study undertaken by our team in the pollination season of 2002 (the study group was different) the first nasal symptoms appeared when the pollen count reached the level of 20 grains/m³ of the air. However, the threshold value for conjunctival symptoms was found to be 50 grass pollen grains/m³ [17]. The scarcity of reports concerning the aforementioned issue, and sometimes conflicting results available in the literature, warrant further studies with larger cohorts of patients.

The correlation between the level of reactivity in CPT and conjunctival symptoms during grass pollination season that we observed in our studied group, has its clinical implications. The conclusions that can be drawn from patient's reactivity in CPT seem to be very helpful, both in treatment recommendations and specific immunotherapy planning (both pre-seasonal and perennial).

CONCLUSIONS

Based on the results of our study, we conclude that the patients with pollinosis and high reactivity in the conjunctival provocation test develop conjunctival symptoms earlier during the grass pollination season (at lower levels of grass pollen count) than the patients who are characterised by the lower reactivity during the conjunctival provocation test (reacting to the allergen extract of the higher concentration). However, no statistically significant correlation was found between the sequence of the symptoms appearance during the conjunctival provocation test with the grass pollen allergen, and the sequence of symptoms appearance during grass pollen season in patients with pollinosis.

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