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"Research Note"

ALLERGENECITY AND IDENTIFICATION OF SPECIFIC IgE BINDING PROTEINS IN POLLEN OF *SPARTIUM JUNCEUM* L. (FABACEAE) AND *LAGERSTROEMIA INDICA* L. (LYTRACEAE): THE EFFECT OF AIR POLLUTION ON THEIR ALLERGENECITY*

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Abstract – Air pollutants are reported to increase the specific IgE response to allergens. To study allergen proteins and determine the effect of air pollutants on the allergenic potential of pollen extracts, the extracts were injected intraperitoneally and subcutaneously in guinea pigs. The results were analyzed by skin prick test (SPT), ELISA and SDS-PAGE followed by immunoblotting. The pollen extract of *Spartium junceum* showed positive SPT and increase of specific IgE antibodies than the control (buffer). Two IgE-binding bands were seen in the immunoblots of the pollen extract of this species. In *Lagerstroemia indica*, the pollen extracts showed no significant difference in wheel diameter compared to those in the control group, but IgE somewhat increased in the pollen extracts. The pollen extracts collected from the polluted region increased the response of SPT and specific IgE in both species. These extracts did not affect IgE-binding bands (proteins). Therefore, these findings indicate that air pollutants show adjuvant activity for the response of SPT and the production of IgE antibodies in guinea pigs by themselves.

Keywords – Air pollution, allergenecity, *Lagerstroemia indica*, pollen, *Spartium junceum*, specific IgE binding proteins

1. INTRODUCTION

Pollen is a natural part of the air flora in all seasons, but is especially numerous in spring and early summer in temperate climates when susceptible humans, breathing the pollen, develop the symptoms of hay fever and asthma [1, 2]. The number of patients who are allergic to pollen has increased during recent years, and it has been assumed that air pollutants could play a role, perhaps by affecting allergens or merely by being transported on the surface of the pollen grain [1-3]. Also, biological aerosols carry antigenic proteins released from pollen grains [3]. There have been some reports suggesting that IgE antibody production and air pollution are related [4] but there has been no evidence to show this directly [5]. A most likely explanation would be that pollutants increase the permeability of the respiratory mucosa, thus affecting humans directly. Experiments on guinea pigs have shown that Ozone, No_2 and So_2 all increase the risk of allergic sensitization [6]. Popp et al. and Traidl-Hoffmann et al. suspected that increasing levels of air pollution could undermine local defense barriers and increase mucosal hypersensitivity [3, 7] Diesel exhaust particles stimulate the synthesis of IgE and cytokines and thus facilitate the allergic sensitization of predisposed subjects [8, 9]. Air pollution, particularly from

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particulates emitted by vehicle emissions and diesel engines, may play a role because they could act as an adjuvant for the antigen of pollen allergy [10]. This study highlights allergenecity and identification of allergens in two ornamental plants of *Lagerstroemia indica* (crepe myrtles) and *Spartium junceum* (spanish broom) that have distributed widely in parks and landscapes. Also, the importance of the exposure to a combination of particulate matter and pollen allergens in the induction of allergic diseases are studied.

2. MATERIALS AND METHODS

Pollen grains were collected randomly from *Lagerstroemia indica* and *spartium junceum* plants grown in a control area (National Botanical Garden, Paykanshahr, Tehran, 30 km far from Tehran) and from plants grown in a polluted area with heavy traffic (the city centre) in June 2002. The climatic and edaphic conditions in both regions were the same. Reports by the air quality centre at the Environment Protection Agency of Tehran showed the type and mean of air pollutant concentrations in both the control and polluted areas at the sampling sites (Table 1). To determine the effect of air pollution on allergenecity and allergens, pollen grains extracts were prepared by incubating in 0.1M phosphate buffered saline, PBS, and pH 7.4 in a 15% ratio with stirring at 4-8 ^{o}C for 3-4 hrs. Suspensions were centrifuged at 10000 g for 40 minutes and supernatants were removed [11]. Guinea pigs were injected on three occasions at weekly intervals through the intraperitoneal route with 200 µl pollen extract containing 75 µg protein, both in polluted extracts and non polluted ones. Blood was collected through periorbital bleeding at 2 days before the first immunization. At day 35, guinea pigs were sacrificed and the blood was collected by cardiac puncture. Sera were obtained by centrifugation and stored at -20 ^{o}C until use [5, 6]. The skin prick test (SPT) was done by intracutaneous injection of pollen extracts. Standard positive (histamine) and negative (buffer) controls were used. Skin tests were read and results were recorded at 15 and 30 minutes. The definition of a positive skin test required a wheal diameter 3 mm or greater (≥) than the salin e control [12]. Five animals were used for each treatment. Statistical analyses were performed using ANOVA and Dunkan's test. Results with $p < 0.05$ were considered significant. All serum samples were also studied by an IgE enzyme linked immunosorbent assay (ELISA). Proteins from samples and molecular markers (Serva, Feinbiochmica Gmbh and Co.) were separated using SDS- PAGE [13]. The proteins were then stained with coomassie brilliant blue. The unstained gels were electrotransferred to PVDF membrane for Western analysis [14]. The membranes were incubated with the patients' sera. The IgE-binding proteins were revealed with an enzyme system using an anti-IgE peroxidase conjugate. In this method, the pattern of molecular weights was revealed with amido-black [14].

Table 1. Mean of air pollutants concentration in control and polluted areas

Type of air pollutant / Month	SO2, ppm	NO2, ppm	CO, ppm	HC (hydrocarbons), ppm	APM (Airborne particulate material), µgm-3
June (polluted area)	0.063	0.06	9.1	2.8	162
June (control area)	0.002	0.01	0.6	0.1	54

3. RESULTS

Skin prick tests response to pollen extracts was higher in polluted and non polluted pollen extracts in comparison to the buffer in *S. junceum*. This response was higher in polluted pollen extracts than non polluted ones (Fig. 1). No significant difference in wheal diameter was observed with sera from animals

immunized with the pollen extract of *L. indica* and buffer, but wheal diameter was bigger in guinea pigs injected with polluted pollen extracts than that seen in non polluted pollen extract and buffer (Fig. 1). Specific IgE antibodies were found in the serum of guinea pigs treated with pollen extracts in all samples. IgE antibody response was higher in guinea pigs immunized with polluted pollen extracts than in animals immunized with non polluted extracts suggesting adjuvant effect of air pollutants (Fig. 2). Immunoblot of IgE binding to *S. junceum* proteins is shown in Fig. 3. Both polluted and non polluted extracts revealed two bands in the ranges 46-55 kDa and 35 kDa. The Immunoblot profile of *L. indica* did not reveal any clear IgE-binding protein band, although in a few of these blots a weak band in non polluted pollen extracts (control extracts) was observed.

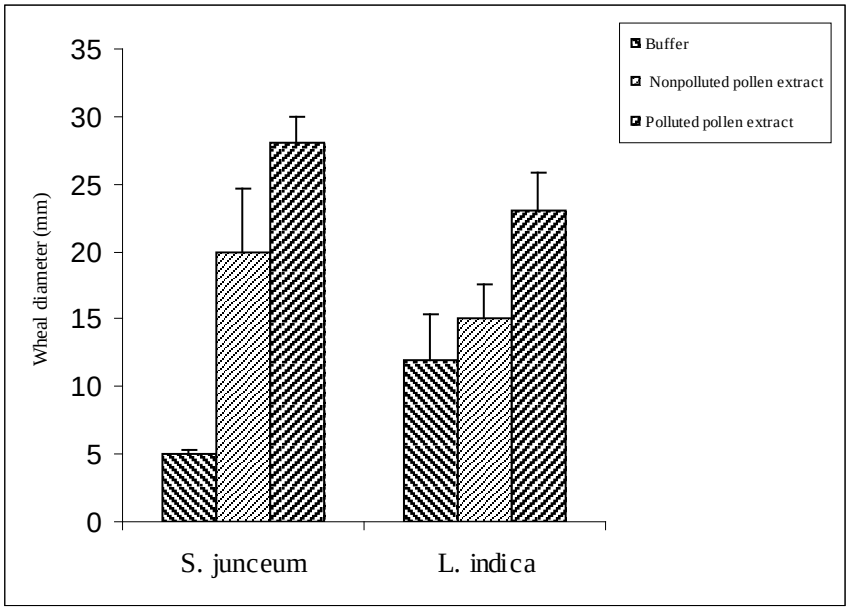

Fig. 1. Prick skin test reactivity in guinea pigs immunized with pollen extracts. Wheal diameter (mm) (mean ± standard error)

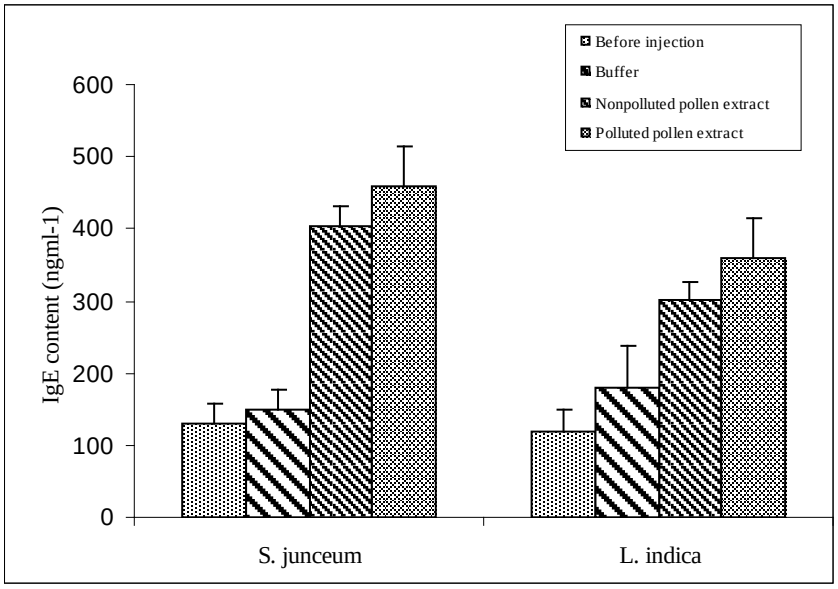

Fig. 2. ELISA reactivity of sera from guinea pigs immunized to pollen extracts. Adjuvant effect of air pollutants on IgE production is obvious. IgE ($ngml^{-1}$) (mean ± standard error)

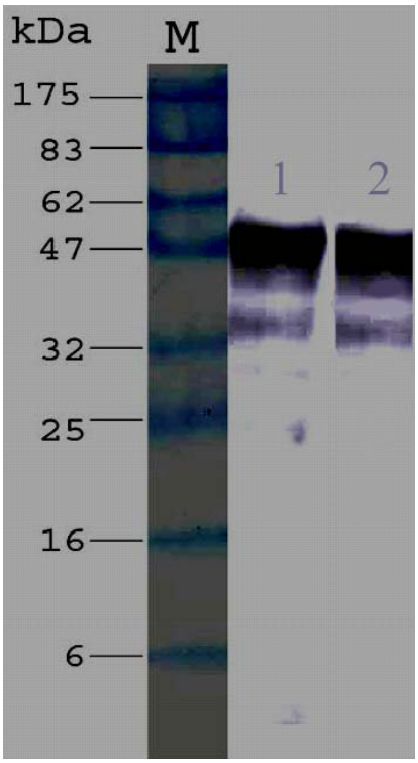

Fig. 3. Immunoblot profile of the pollen extracts. M: Marker; 1: Non polluted pollen extract; 2: Polluted pollen extract

4. DISCUSSION

In this study, pollinosis was seen in *Spartium. junceum* and *Lagerstroemia indica*. Wheal diameter in animals immunized with the pollen extracts of *Spartium junceum* increased significantly in comparison with the control (buffer), whereas this difference was not significant in *Lagerstroemia indica* ($P < 0.05$). A significant increase ($P < 0.05$) of PST response was observed in the pollen extracts collected from polluted areas than those from non polluted ones, indicating the role of air pollutants in allergenecity. The guinea pigs presented a specific IgE antibody through the pollen extracts of these species. Specific IgE titres increased in guinea pigs immunized and challenged with pollen extracts collected from polluted areas. These results are consistent with the results of other researchers such as [3-5, 9, 14-17]. These researchers have reported that pollen can collect pollutants on its surface during its many hours of travel on the air flow. These pollutants could be toxic in themselves, or they could cause disorders by enhancing the allergenic properties of the pollen's surface proteins. Therefore, there is growing evidence that air pollutants act as adjuvants in the immune system and lead to enhancement of allergic inflammation. Nel and Diaz-Sanchez showed air pollutants enhance IgE production by a variety of mechanisms, including effects on cytokine and chemokine production, as well as activation of macrophages and other mucosal cell types [18]. IgE-binding bands did not show any obvious difference between polluted and non polluted extracts in *S. junceum*. In *L. indica*, no unique IgE-binding protein band was observed. Studies on pollen proteins show contradictory results. Our results are consistent with the results of Helender et al. [19] who observed no significant difference between the protein bands of polluted and control areas. However, studies of Behrendt et al. showed a dose-dependent shift in the intensity of IgE binding reactivity to lower molecular weight bands [20]. In addition, Hjelmroos et al. [21] and Parui et al. [22] found a decrease in Bet v 1 concentration under air pollution. It is possible that the type of plant species could easily be the cause for these differences, and therefore, this matter may need to be studied further.

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