

# Occupational Asthma, Eosinophil and Skin Prick Tests and Serum Total IgE Values of the Workers in a Plant Manufacturing Rose Oil

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Although occupational air pollution and allergen exposure have long been known as causes of occupational asthma, the incidence and prevalence of the asthma have not yet been vigorously investigated in many countries.<sup>1</sup> The prevalence of asthma induced by the exposure to some plant pollens including red cedar and some occupational agents such as TDI (toluene diisocyanate) has been estimated to be around 15%. Since asthma is a disorder commonly diagnosed at the adult ages the relationship between the initiation of asthma and exposure to a specific asthma causing agent at the working place has frequently been investigated. Up to 1994, over 250 specific asthma causing agents have been identified and the list of occupational asthma (OA) causing agents has expanded year to year.<sup>2-4</sup> Workers for beer, baking and plastic manufacturing plants have often

**SUMMARY** This study was aimed to determine the rate of occupational asthma (OA) in workers at a rose extracting plant. Specific clinical tests of 52 workers, randomly chosen from four local rose extracting plants, were statistically compared with the test results of 30 local control subjects of similar age and sex as the plant workers, but who had never worked in such a plant. There were no significant differences in pulmonary function tests (FVC, FEV1, FEV1/FVC, PEFr) between the control and test groups. Significantly higher serum total IgE values ( $p < 0.0001$ ) were observed for the test subjects ( $239.08 \pm 240$  IU/ml) compared to the control subjects ( $81.33 \pm 61.45$  IU/ml). There were also significant differences ( $p < 0.0001$ ) in the number of eosinophils between the control and test groups, with corresponding mean values of  $2.28 \pm 2.75\%$  and  $0.73 \pm 1.72\%$ , respectively. A specifically prepared skin prick test using a rose allergen (*Rosa domescena*) was positive for 53.84% in the test subjects whereas only 5.33% positive test results were seen in the control group. We have demonstrated the involvement of *Rosa domescena* pollen in occupational allergy, through IgE-mediated hypersensitivity. It was concluded that the workers of a rose oil extracting plant are more susceptible to the rose pollens.

been found to suffer from OA.<sup>5-7</sup> The rose has been cultivated by a small number of countries, mainly Turkey and Bulgaria. In Turkey, Isparta is the main rose cultivating region and with a concentration of almost all rose extracting plants of the country. Rose oil is removed by syringe and kept in dark glass bottles. This process requires patience as

only one gram of oil is produced from four kilograms of rose petals.

Rose oil is commonly used in the cosmetic industry as a raw ma-

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terial. The workers of rose oil extracting plants are repeatedly exposed to rose pollens and environmental dusts from May to July every year, and such are at risk of occupational asthma. Therefore, we intended to determine the OA for the workers exposed to rose pollens through a questionnaire specific to asthma symptoms, through respiratory functional tests and skin prick tests and by determining total serum IgE and eosinophil levels.

## MATERIAL AND METHODS

The study was conducted on 52 workers randomly chosen from four rose extracting plants and 30 healthy subjects not working in such a plant. The study group only included patients who did not have a systemic disease, infections (such as, bronchitis, sinusitis, etc.) or a history of atopy. All patients were duly informed about the study and only those who gave their informed consent were included. A standardized asthma questionnaire including questions on occupational issues taken from previous studies was completed by every subject together with a physician. A diagnosis of rhinitis was made if a worker noted rhinorrhea, nasal congestion, and/or sneezing. Conjunctivitis was determined by the presence of ocular itching, burning, or tearing of the eyes. Occupational rhinitis and/or conjunctivitis required the presence of symptoms only at work.<sup>8,9</sup> The study was done during the harvest season in May, June and July 2002.

### Pulmonary function tests

The pulmonary function tests were performed using a spirometer (SCHILLER SP-10, Swiss).

These tests were assessed according to the spirometer's manual. Additionally, each of the respiratory function parameters of the patients was tested twice and the best value was accepted for the study. The test values were recorded four times a day for 4 days by the Personal Best, Full Range Peak Flow Meter (USA) device, and diurnal change was calculated as the mean of all tests from the highest and lowest values.

### Bronchial hyperresponsiveness (BHR)

Methacholine was diluted in PBS (phosphate buffered saline) at 25 mg/ml. The protocol consisted of one normal inhalation, followed by three courses of four breaths each of the 25 mg/ml methacholine solution. FEV<sub>1</sub> was measured 3 minutes after each inhalation. The exercise was terminated when the fall in FEV<sub>1</sub> value was equal to or higher than 20% of the postdiluent value, or after the third series of four breaths of methacholine was done. Results were expressed in terms of the cumulated methacholine units (one methacholine unit = one inhalation of the 1 mg/ml concentration) needed to decrease FEV<sub>1</sub> by 20% from the postdiluent value (M-PD<sub>20</sub>).

### Skin prick test

Allergen extracts were used for the test (Standardized extract; 10,000 AU/ml; Center Laboratories, Port Washington, NY-11050). The extracts contained 1) mite-I (*D. pteronyssinus*), 2) mite-II (*D. farinae*), 3) Special grass mix, 4) Special tree mix, 5) Special mold mix, 6) Special weed mix, respectively. Additionally, a rose allergen stan-

dardized in a French laboratory (Stallergenes) was used. Histamine (Histatrol: histamine base 1 mg/ml, histamine phosphate 2.75 mg, contained in 50% glycerin) was used as positive control, and saline solution as negative control. All the above tests were performed using a multiple skin test applicator (Multitest II, Lincoln Diagnostics, Inc., Decatur, Ill., USA). The results of the skin prick tests were read after 20 minutes, and only a wheal with a flare larger than 5 mm was considered a positive result.

### Total IgE test

Five milliliters of the blood samples obtained from all subjects were centrifuged at 1,000 x g for 5 minutes for serum collection. The serum samples were kept at -20°C until total IgE analysis. Total IgE analysis was performed at the Department of Microbiology of the Süleyman Demirel University Hospital using a commercial kit (Behring, Marburg, Germany) through a BN ProSpec System (Behring) device according to the method of immuno-nephelometry.

### Eosinophil analysis

Two milliliters of each blood sample were dropped into tubes containing EDTA. The samples were brought into the hematology laboratory of the Süleyman Demirel University Hospital to be analysed in an ADVIA-120 device using a commercial kit (Beckman, Miami, USA).

### Statistical analysis

All data were statistically analysed through the computer program "SPSS 9.00 for Windows"

using the tests of Mann-Whitney U and chi-square. Significant levels were set at  $p < 0.05$ .

## RESULTS

The present study was carried out on a total of 82 human subjects, 52 of whom were test workers exposed to rose pollens and 30 of whom were the control group consisting of non-workers who were never exposed to rose allergens. The test group comprised 22 females with an average age of  $26.84 \pm 4.32$  years and 30 males with an average age of  $28.98 \pm 8.42$  years. The control group comprised 10 females with an average age of  $25.38 \pm 4.12$  years and 20 males with an average age of  $27.66 \pm 5.16$  years. All of the workers had all worked in the rose plants for a period of 3 months every year for at least 3 years. The results of the standard asthma questionnaire did not reveal any significant differences in asthma symptoms between

the subject workers and the control group ( $p > 0.05$ ) (Table 1). However, 53.84% of the test workers positively responded to the skin prick test done with rose allergen (*Rosa domescena*) whereas 5.33% of the control subjects responded positively (Table 2).

methacholine was negative in the control subjects while workers (24 males and 18 females) responded positively (Table 3).

The results of the pulmonary function tests (force vital capacity, FVC; force expired volume; FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, and peak expiratory flow rate, PEFR) total IgE and

The BHR test done with

**Table 1** Rose-related asthma and allergic symptoms during the rose season

Asthma and allergic symptoms	Working group	Control group
Chest tightness	1	-
Shortness of breath	1	-
Cough	2	1
Wheeze	-	-
Asthma attack	-	-
Nose symptoms	2	1
Eye symptoms	1	1
Itching hands	1	-

**Table 2** Rose plant workers and control group skin prick test and total serum IgE results

Allergen	Groups	n	Positive (%)
<b>Dermatofagoides pteronyssinus</b>	Workers	52	34 (65.38)
	Controls	30	14 (46.66)
<b>Grass mix</b> (Bermuda grass, rye grass, timothy, orchard grass, june grass, sweet vernal, meadow fescue, red top)	Workers	52	39 (75)
	Controls	30	16 (53.33)
<b>Tree mix</b> (Birch, olive tree, W poplar, hazelnut, W oak, W elm, W ash, alder)	Workers	52	35 (67.30)
	Controls	30	14 (46.66)
<b>Weed mix</b> (Mugwort, lamb quarters yellow dock, short ragweed, dandelion, wall pellitory, quak grass)	Workers	52	32 (61.53)
	Controls	30	13 (43.33)
<b>Mold Mix</b> ( <i>Alternaria alternaria</i> , <i>Aspergillus fumigatus</i> , <i>Cladosporium</i> spp., <i>Candida albicans</i> )	Workers	52	6 (11.53)
	Controls	30	3 (10)
<b>Rosa domescena</b>	Workers	52	28 (53.84)
	Controls	30	2 (5.33)

eosinophil levels are presented in Table 4. It can be seen clearly from Table 4 that there were no significant differences ( $p > 0.05$ ) between the control and test groups. In contrast, total IgE and eosinophil levels were significantly different between the control and test groups, ( $p < 0.0001$  and  $p < 0.0001$ , respectively).

## DISCUSSION

Plants are probably the most common source of occupational allergens, mainly different types of wood, cereal flours, coffee beans, latex, plant food, and enzymes.<sup>10</sup> Seasonal workers of rose extracting plants are intensively exposed to rose allergens and pollens and intermediate agents during the processing. Possible respiratory symptoms induced by those agents were identified by a OA questionnaire which included occupational questions. However, the symptoms did not differ between the control and

the study group ( $p > 0.05$ ). Studies on the worker's susceptibility and sensitization to rose allergens are rare. High-molecular-weight agents (e.g., animal proteins and flour) act as complete antigens and induce the production of specific IgE antibodies. The rate for acquiring sensitization and symptoms may differ according to the nature of the agent. Sensitization to work-related allergens occurs during the first 2 years in apprentices exposed to laboratory

animals but not in apprentice bakers. Moreover, in laboratory-animal workers, the incidence of rhinoconjunctivitis symptoms is greater in years 1 and 2 after starting the exposure, whereas the incidence of respiratory symptoms is greater in years 2 and 3. Rhinoconjunctivitis symptoms generally precede the onset of asthma symptoms in the case of high-molecular-weight, but not low-molecular-weight, agents.<sup>11</sup> However, the workers did not exhibit

**Table 3** Methacholine challenge test results

Patients no.	Methacholine PD <sub>20</sub> values (methacholine cumulated units, mcu)
4	5.78 - 12.0
7	12.1 - 24.5
18	24.6 - 49.5
11	49.6 - 99.5
2	99.6 - 225
10	Negative

**Table 4** Respiration function tests, total serum IgE and eosinophil values of the rose plant's workers (test) compared with control group

Parameters <sup>1</sup>	Groups	N	Mean ± SD <sup>2</sup>	T test	p
FVC	Workers	52	4.044 ± 0.832	0.466	> 0.05
	Control	30	3.962 ± 0.618		
FEV <sub>1</sub>	Workers	52	3.433 ± 0.896	0.141	> 0.05
	Control	30	3.407 ± 0.622		
FEV <sub>1</sub> /FVC	Workers	52	83.236 ± 13.478	-0.674	> 0.05
	Control	30	85.166 ± 10.514		
PEFR	Workers	52	7.094 ± 2.929	0.119	> 0.05
	Control	30	7.016 ± 2.716		
Total IgE	Workers	52	239.082 ± 240.497 IU/MI	3.518	< 0.0001
	Control	30	81.333 ± 61.453 IU/MI		
Eosinophils	Workers	52	2.288 ± 2.753%	2.791	< 0.0001
	Control	30	0.733 ± 1.720%		

<sup>1</sup>FVC, force vital capacity; FEV<sub>1</sub>, force expired volume; FEV<sub>1</sub>/FVC; PEFR, peak expiratory flow rate.

<sup>2</sup>Mann-Whitney U test

a bronchial asthma to rose pollen allergens, but atopic dermatitis symptoms and rhino-conjunctivitis symptoms. Recently, Unlü *et al.*<sup>12</sup> have described *Rosa domescena* factory workers suffering from occupational asthma who were sensitized to *Rosa domescena* pollen. We also did not find any asthma attack among the workers due to sensitization to *Rosa domescena* allergens. Symptoms due to rose mainly affect the upper airways. A negative histamine or methacholine challenge test result does usually not exclude occupational asthma if performed when the patient is off work and free of symptoms. However, if the challenge test is performed when the patient is working and symptomatic, the diagnosis of OA can be excluded.<sup>11</sup> We did not find any difference for each of the lung function tests (FVC, FEV<sub>1</sub>, FEV<sub>1</sub>/FVC, PEFr) between the 2 groups, whereas there was a significantly increased BHR in the study group compared to the control group ( $p < 0.0001$ ). Most studies in the OA literature have focused on antibody-dependent or antibody-mediated reactions.<sup>13</sup> Antigen-specific IgE seems to be more common in OA caused by sensitization from high-molecular-weight proteins.<sup>14</sup> The ability of occupational agents to induce an allergic disease is influenced by the physical-chemical properties of these substances, their dose, exposure duration and route, as well as other factors such as industrial hygiene. Regarding the sensitization to rose allergens, total serum IgE and eosinophil levels were significantly higher in the study group than the control group ( $p < 0.0001$  and  $p < 0.0001$ , respectively). Although the mechanisms by which

many occupational agents may induce asthma are largely unknown, an established IgE-mediated sensitization has been demonstrated for some of these agents. Sanchez-Guerrero *et al.*<sup>15</sup> and Alvarez *et al.*<sup>16</sup> demonstrated that oilseed, rape and flour sensitization (skin tests and BHR) were detected in all patients. An association of specific IgE with atopy, other pollen allergy and seasonal rhinitis is also consistent with the possibility of IgE-mediated allergic reactions to rose.<sup>17</sup> Nevertheless, a positive skin prick test and BHR test indicated that there was a greater degree of sensitization to rose pollens despite the fact that none of the workers exhibited a clinical symptom of bronchial asthma. In this study only 53.8% had a positive skin prick test to a rose allergen whereas up to 80% had BHR positive. As a result BHR and skin prick test were found more sensitive than allergy symptoms for rose allergens. Literature has rare scientific findings indicating the relationship between the sensitization to rose pollens and bronchial asthma.

In summary, we have demonstrated the involvement of *Rosa domescena* pollen in occupational allergy by IgE-mediated hypersensitivity; therefore the inhalation of material derived from *Rosa domescena* pollen may cause respiratory allergy in exposed workers.

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