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Fungal Flora at Solid Waste Storage Centres and Their Potential Allergenic Effects on the Workers

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Key Words

Moulds · Fungi · Solid waste storage centres · Allergy · Prick test

Abstract

Purpose: The aim of this study was to find out the fungal flora at solid waste storage centres and its potential allergenic effects on the workers.

Methods: Air samples from 10 solid waste storage centres were collected and plates were incubated for up to 14 days. The isolated fungi were identified by standard methods. In total, 599 solid waste sanitation workers who worked in those areas were questioned about nasoconjunctival and skin allergic symptoms and then underwent skin prick tests with different mould extracts.

Results: *Aspergillus* sp., *Cladosporium* sp., *Rhizopus* sp., *Penicillium* sp., *Alternaria* sp. and *Trichoderma* sp. were isolated in all areas, *Aspergillus* being the most abundant mould followed by others. According to skin prick tests, positivity to *Aspergillus fumigatus* was found to be in the first order by 10.68%. There was a significant correlation between the nasal and conjunctival symptoms and skin test positivity to house dust mites ($p = 0.002$, $p = 0.03$). Skin complaints were correlated with skin test positivity to *Penicillium notatum* ($p = 0.04$).

Conclusion: Our findings showed that the workers of solid waste storage centres are under a moderate risk of becoming sensitised to some mould species, especially to *Aspergillus* sp. The environmental measurements and periodical medical check-ups must be taken into

account in solid waste storage centres in order to assess the health risks.

Introduction

People in some occupations are more exposed to mould and at greater risk of developing allergies. Farmers, bakers, mill workers, carpenters, greenhouse employees, wine makers, furniture repairers, garbage collectors and solid waste sanitation workers are at increased risk [1]. The risks coming from the worker's social life should be taken into account in addition to the hazards of the working environment. Solid waste storage and its maintenance is a high-risk sector covering all hazards mentioned before. There are fungi, bacteria and viruses which are classified as biological factors coming from work environment [2]. These air pollutants directly affect the respiratory and cardiovascular systems; the severity of the effect varies according to both intensity and duration of exposure as well as the health status of the population exposed to pollution [3].

Allergy to moulds has been known for several decades. Residential or office fungal exposures may be a substantial factor in developing an allergic airway disease depending on the subject's allergic sensitivity and the levels of indoor exposure [4]. Exposure to moulds in several environments including industrial fields is an important factor than can stimulate allergic diseases. Respiratory diseases caused by inhalation of mould spores, such as allergic rhinitis, atopic asthma, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis and allergic fungal sinusitis are well recognised [5]. In solid waste storage centres, physical and biological factors including moulds may lead to a rise in upper and lower respiratory or skin complaints precipitating allergenic sensitivity. The aim of this study is to find out the fungal flora at solid waste storage centres and its potential allergenic effects on the workers.

Materials and Methods

Sampling

This cross-sectional study took place in Istanbul. It involved 599 solid waste sanitation workers who worked in 10 different storage areas. The purpose of the study was explained to the study participants and their approval was

received beforehand. The study was carried out between October, 2006 and February, 2007.

Questionnaire

In order to determine the health status of 599 individuals, the following information was obtained through face-to-face interviews: their ages, smoking habits, previous jobs, any history of a serious disease, and whether they had previously worked in smoke or dust filled workplaces, whether they had pulmonary disease or suffered from one in the past and whether they had had allergies. We also asked if they ever coughed, if there was phlegm while coughing and whether they had shortness of breath, a stuffy nose, red, itchy and watery eyes, skin rash, eczema, dermatitis or hives. Three questions concerning nasal allergies, allergic conjunctivitis and itching dermatitis, urticaria and eczema were adapted from European Community Respiratory Health Survey Questionnaire stage-I [6].

Control of Air Quality in the Open Areas

For the bio-aerosols, a Merck Air Sampler 100 (MAS 100) (Merck KGaA-64271, Darmstadt, Germany) operated at 100 L min^{-1} of air in combination with Dichloran 18% Glycerol (DG-18) agar and Malt Extract Agar (MEA) was used to collect airborne fungal propagules [7,8]. During sampling, the MAS 100s were placed at approximately 1 m above the floor level and calibrated before use. To eliminate the possibility of contamination, three Petri dishes with MEA, DG-18 agar and plate count agar were also incubated for the same duration without air sampling. After sampling, the agar plates were removed from the sampler and covered with lids. The agar plates were hand-carried to the laboratory. The plates for the fungi were incubated for up to 14 days at 22°C and 90% relative humidity. The isolated fungi were identified by standard methods [9–11].

Concentrations were calculated as colony-forming units per cubic metre (CFU m^{-3}) of air using the MAS 100 unit conversion table. The rate of aspiration was arranged to be 100 L/min, taking samples from the environment. Petri boxes were used in sampling and they were taken from 10 different points in each region. The assessment used 100 Petri in total by taking 10 samples from 10 different points.

Skin Prick Tests

The sensitivity to common allergenic moulds was determined with skin prick tests. All the subjects underwent skin prick tests separately with the extracts of

Alternaria alternata, *Aspergillus fumigatus*, *Penicillium notatum*, mould mix-1 (*A. alternata*, *Chaetomium globosum*, *Cladosporium fulvum*, *Cladosporium herbarum* and *Fusarium moniliforme*), mould mix-2 (*Mucor mucedo*, *Neurospora sitophila*, *Pullularia pullulans* and *Rhizopus nigricans*), mould mix-3 (*Aspergillus amstelodami*, *A. fumigatus*, *Aspergillus niger* and *Aspergillus terreus*), mould mix-4 (*Penicillium brevicompactum*, *Penicillium expansum*, *P. notatum* and *Penicillium roqueforti*; ALK ABELLO, Madrid, Spain), as well as mites including *Dermatophagoides farinea*, *Dermatophagoides pteronyssinus*, *Blumia tropicalis* (ALK ABELLO, Madrid, Spain), positive control (histamine) and negative control (saline) (ALK ABELLO, Madrid, Spain). Prick tests were regarded as positive when wheal diameter was at least 3 mm and equal to 60% of that induced by histamine.

Statistical Analysis

The evaluation of continuous variables such as RF, age, working years, etc. which were distributed normally in the working groups was made and presented in the tables as the mean and standard deviation (SD), whereas nominal variables were expressed as a percentage. Non-continuous variables were evaluated according to skin prick test using a chi-square test. Statistical significance was accepted as $p < 0.05$ for the results that were two-tailed.

Results

The study group of 599 workers consisted of 599 (100%) males. Details regarding gender, age, height, weight, body mass index (BMI), working period and length of smoking are given in Table 1. The mean age of the workers involved in the study was 38.52 ± 8.15 and their average working year was 4.78 ± 3.90 . The rate of the participants who ever smoked was 54.92% and those who were still smoking 55% (329). Among the smokers, the mean number of the cigarettes and the mean year of cigarette smoking were 20.21 ± 10.12 and 17.69 ± 6.20 , respectively.

Table 1. Age, height and BMI values of the persons taken into the study

Variables	n	Mean	SD	Minimum	Maximum
Age (year)	599	38.52	8.15	17	67
Height (cm)	599	172.60	7.00	150	198
Weight (kg)	599	79.18	12.96	50	125
BMI (kg m^{-2})	599	26.60	4.21	16.98	39.55
Daily working time (h)	599	9.18	1.40	8.00	12.00
Working period (year)	599	4.78	3.90	1.00	22.00

Clinical Evaluation

The rates of the workers who had frequent nasal symptoms, conjunctival symptoms and skin complaints were 30.9%, 36.1% and 30.05%, respectively. The rate of doctor-diagnosed asthma was 2.8%.

Evaluation of Microbiological Findings in Relation to the Environment

For the microbiological evaluations, microorganisms were collected in all Petri dishes that were opened in 10 different areas. They were assessed over 100 Petri in total by taking 10 samples from 10 different points. The frequencies of fungal species detected in the areas of the study are given in Table 2.

The distribution of airborne fungal propagule concentrations in CFU m^{-3} in the study is given in Table 3.

Microorganisms were reproduced in different areas at different rates. In all Petri boxes, *Aspergillus* sp., *Cladosporium* sp., *Rhizopus* sp., *Penicillium* sp., *Alternaria* sp. and *Trichoderma* sp. were isolated in all areas.

According to skin prick tests, positivity to *A. fumigatus* was found to be in the first order by 10.68% ($n = 64$), followed by mould mix-2 8.68% ($n = 52$), *D. farinea* 8.68% ($n = 52$) and others. Susceptibilities to two different moulds were found to be 8.68% and 5.18% of the subjects that were sensitised to three different moulds. The results of skin prick tests are shown in Table 4.

Table 2. The frequencies of fungal species detected in the areas of the study groups

Fungal species	Frequency (%)
<i>Aspergillus</i> sp.	78.80
<i>Cladosporium</i> sp.	12.15
<i>Penicillium</i> sp.	5.50
<i>Alternaria</i> sp.	4.43
<i>Rhizopus</i> sp.	2.77
<i>Trichoderma</i> sp.	1.11
<i>Fusarium</i> sp.	0.96

Table 3. The distribution of airborne fungal propagule concentrations in CFU m⁻³ in the study areas

Species	Mean	SD	Median	Minimum	Maximum
<i>Aspergillus</i> sp.	16,226.70	12,282.47	26,280	20	26,280
<i>Cladosporium</i> sp.	126.38	163.41	75	10	1020
<i>Rhizopus</i> sp.	75.00	112.99	30	10	530
<i>Alternaria</i> sp.	42.61	52.50	20	10	260
<i>Trichoderma</i> sp.	23.33	13.66	20	10	40
<i>Penicillium</i> sp.	22.50	12.82	20	10	40
<i>Fusarium</i> sp.	10.00	0.00	10	10	10

Table 4. The rates of positive skin prick tests

Species	Frequency (n)	Percent (%)
<i>Aspergillus</i> moulds	64	10.68
Mould mix-2	52	8.68
<i>D. farinea</i>	52	8.68
<i>D. pteronyssinus</i>	51	8.51
Mould mix-4	41	6.84
<i>B. tropicalis</i>	41	6.84
Mould mix-1	40	6.68
<i>A. fumigatus</i>	35	5.84
<i>A. alternata</i>	25	4.17
<i>P. notatum</i>	25	4.17
<i>C. herbarum</i>	18	3.00
<i>Mucor mucosus</i>	6	1.00

Table 5. The relationship between the environmental mould measurements and skin test results

Mould species detected in ambient air		Skin test results with mould mix-4				Significance	
		Negative			Positive		
		Number	Percentage		Number		Percentage
<i>Penicillium</i> sp.	Not existing	459	82.26	26	63.41	0.003*	
	Existing	99	17.74	15	36.59		
<i>Trichoderma</i> sp.	Not existing	511	91.58	32	78.05	0.004*	
	Existing	47	8.42	9	21.95		
<i>Aspergillus</i> sp. + <i>Penicillium</i> sp.	Any	459	82.26	26	63.41	0.006*	
	Both	99	17.74	15	36.59		
<i>Penicillium</i> sp. + <i>Rhizopus</i> sp.	Any	459	82.26	26	63.41	0.006*	
	Both	99	17.74	15	36.59		

* $p < 0.01$; values written in italics are statistically significant.

The skin test positivity to *Alternaria* correlated with the working duration in that work.

Environmental Measurements and Skin Prick Test Analyses

Significantly higher skin test reactivity to mould mix-4 extract was found in the workers of the centres, where *Penicillium* sp. or *Trichoderma* sp. was the only fungi grew in the environmental samples. Also, if *Aspergillus*

sp. + *Penicillium* sp. or *Penicillium* sp. + *Rhizopus* sp. grew together predominantly in the samples, mould mix-4 reactivity was significantly higher in the workers of that workplace. Comparison of the environmental mould measurements and skin test reactivities is shown in Table 5.

There was a significant correlation between the nasal and conjunctival symptoms and skin test positivity to house dust mites ($p = 0.002$, $p = 0.03$). Skin complaints

were correlated with skin test positivity to *P. notatum* ($p=0.04$). There was no correlation between the working duration and the existence of clinical symptoms.

Discussion

Our findings showed that the workers of solid waste storage centres are under a moderate risk of becoming sensitised to some mould species, specifically to *Aspergillus*. The only mould species that was found related to allergic skin complaints was *Penicillium*. Similarly, in one study conducted on subjects with allergic rhinitis, Gelincik et al. [12] found that only the counts of *Penicillium* spores among environmental fungi was found to be significantly correlated with skin test positivity.

Although the comparison with the healthy control group is lacking in this study, it is generally accepted that mould hypersensitivity is around 5% in population [13]. However, we found that skin sensitivity to *Aspergillus* was 10.68%, while for many species it was more than 5% in waste storage workers. On the other hand, the rates of work-related nasal and conjunctival symptoms were rather high (30.9% and 36.1%, respectively) among the workers. Although these symptoms correlated only with house dust mite sensitivity, the skin test positivities to mites (Der f Der p) were found to be only 8.68% and 8.51%, respectively. Therefore, we can suggest that the upper respiratory symptoms of workers may be stimulated with exposure to workplace-related bioaerosols as Heldal and Eduard suggested [14].

The most abundant mould species found in solid waste storage centres was *Aspergillus* sp. with the median $26,280 \text{ CFU m}^{-3}$. This finding was consistent with the skin test results which displayed that *A. fumigatus* was the most dominant sensitizer among the workers. Bush and Portnoy [15] stated that indoor spore numbers $\geq 1000 \text{ m}^{-3}$ or colony numbers between 1000 and $10,000 \text{ CFU m}^{-3}$ reflect indoor contamination. Wiszniewska et al. showed

that 19.8% of the subjects who lived in dwellings with evident signs of fungal contamination were sensitised to at least one mould allergen. However, the association between indoor mould exposure and the development of mould allergy was not confirmed in their study [16]. Although we could not demonstrate a correlation between the *Aspergillus* sensitivity and clinical symptoms, a significant correlation was found between the skin symptoms and *P. notatum* sensitivity.

Exposure to moulds is thought to cause adverse health effects ranging from vague subjective symptoms to allergy and respiratory diseases [17]. Some of the fungi in the indoor air are most commonly cited as causes of rhinitis and asthma [18–20], such as *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp. and *Rhizopus* sp. The survey of Palmas et al. [21] showed that fungi can play a significant role in allergic and non-allergic diseases in modern working environments. Among the workers of solid waste storage centres, bad working conditions and gases (methane, hydrogen, sulphur, etc.) dispersed in the environment give rise to an increase in respiratory complaints and precipitate occupational diseases. Although we could not detect, it is expected that other biological factors such as bacteria and above-mentioned chemical factors in the environment may cause nasal and skin symptoms of workers in solid waste storage centres.

In conclusion, the environmental measurements and periodical medical check-ups must be taken into account in solid waste storage centres in order to assess the health risks.

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