

Field Study

Environmental Mycological Study and Allergic Respiratory Disease among Tobacco Processing Workers

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Abstract: Environmental Mycological Study and Allergic Respiratory Disease Investigation in Tobacco Processing Workers: Yongxing ZHANG, *et al.* Division of Pneumoconiosis, School of Public Health, China Medical University, P.R.China—This study presents the results of an investigation of respiratory symptoms, lung function, chest X-ray examinations, analysis of specific IgE antibodies and skin prick tests to fungi on 130 tobacco-processing workers and 112 control workers. Industrial hygiene survey and environmental mycological studies were also performed. The average dust concentrations ranged from 13.76 to 29.55 mg/m³ in the tobacco processing workshops. Also, the numbers of fungi colonies in the processing environments were much higher than those in the control environments. The prevalences of chronic respiratory or nasal symptoms in exposed workers were significantly higher than those in control workers ($p < 0.05$ or $p < 0.01$). The prevalences of lung function abnormalities in the exposed workers were significantly higher than those in control workers ($p < 0.05$). The positive prevalences of specific IgE reactions to fungi (26.92% for *A.fumigatus* and 51.54% for *Rhizopus nigricans*) in exposed workers were also significantly higher than those in control workers ($p < 0.01$). The positive prevalences of the skin prick test showed that 18.46% of the exposed workers were positive to *A. fumigatus* and 23.85% were positive to *Rhizopus nigricans*. Our results suggested that tobacco processing workers may develop respiratory diseases related to tobacco dust and we consider that fungi might be one of the allergens causing allergic respiratory or nasal diseases in tobacco processing workers. (*J Occup Health* 2005; 47: 181–187)

Key words: Tobacco dust, Respiratory symptom, Lung function, Specific IgE to fungi, Skin prick test

Respiratory diseases in tobacco processing workers have been described by other scholars. Viegi¹⁾ reported that respiratory or nasal symptoms in cigar and cigarette making workers were significantly higher than controls. Lander and Gravesen²⁾ found that 69% of tobacco workers had symptoms of occupational asthma and chronic bronchitis. Uitti³⁾ and Mustjbegovic⁴⁾ showed that tobacco workers tended to have lower FVC, FEV₁, FEF₅₀, and FEF₂₅. Gleich⁵⁾ observed that allergy to tobacco products is an occupational hazard and the allergic reactions to tobacco antigens in tobacco leaves might be responsible for the disease. Huuskonen⁶⁾ suggested that exposure to spores of different molds in the manufacture of tobacco products may induce symptoms and signs relating to extrinsic allergic alveolitis. We conducted investigations of respiratory symptoms, chest X-ray examinations, lung function, specific IgE antibodies to fungi, and the skin prick test to fungi on 130 tobacco processing workers and 112 control workers. At the same time, an industrial hygiene survey and environmental mycological studies were also performed. Our objectives were to investigate whether tobacco processing workers develop allergic respiratory or nasal diseases related to tobacco dust or fungi and to discuss the possible causes of the respiratory injury in the tobacco processing factory.

Material and Methods

Subjects: The tobacco processing factory investigated was located in Anshan city Liaoning province, in the northeastern part of China. The tobacco leaves as primary raw materials are processed in the following steps: unpacking, baking, reeling off, blending and wrapping.

A total of 130 workers in the tobacco processing factory were investigated in this study. The four criteria for subject selection were (1) exposure to tobacco dust for at least one year; (2) no exposure to other toxicants; (3) absence of tuberculosis and heart disease; (4) non-smoker. Among the subjects, 70 male workers were 36 yr old on average (36.0 ± 18.6 yr old), and 60 female workers were

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33 yr old on average (33.0 ± 15.0 yr old). The 112 control workers, with no exposure to dust or toxicant, worked in a machine tool processing company and resided in the same city and had similar labor intensity. This ensured that there were no differences in socioeconomic status, work intensity, and exposure to atmospheric pollutants between the control and exposure groups. Among the control subjects, 42 male workers were 35 yr old on average (35.0 ± 15.4 yr old); and 70 females were 34 yr old on average (34.0 ± 11.0 yr old).

Environmental surveys of dust concentration, free silica content and environmental mycological study: The total dust concentration was measured by area sampling, and 2–4 locations for each tobacco processing workshop were selected for sampling. Four samples were collected during the entire 8-h shift, and sampling was continued for 2 d in each location. Air sampling pumps were utilized. The arithmetic means of dust concentrations in all workshops were calculated as time-weighted average (TWAs) according to the data obtained in the surveys. The gravimetric method was carried out to analyze the silica content. Size distribution of the dust was examined using a microscope. Out-plate culture (90 mm) was adopted to inspect the fungus conditions of all workshops in the middle of April, August and December. The culture plates used for sampling atmospheric molds contained Sabouraud's agar medium with gentamicin (50 $\mu\text{g}/\text{ml}$). Five plates were exposed for 5 min in the workers' breathing zone (1–1.5 m high) for each processing workshop. The fungus colonies were counted after being incubated at 25–28°C for 7 d. Species of isolated fungi were identified according to morphology under the microscope.

Respiratory symptom investigation and chest X-ray examination: Data on respiratory symptoms were collected by means of a self-administered Chinese version of the British Medical Research Council Questionnaire on Respiratory Symptoms (BMRC)⁷⁾. Additional questions about allergic symptoms and occupational history were included (The respiratory symptom questionnaire: see Appendix I). The quality of Chest X-ray films was according to the diagnostic standards of X-ray films of pneumoconiosis. Chest X-ray films were read by a qualified pneumoconiosis diagnostic expert to diagnose pneumoconiosis according to "Diagnostic Criteria of Pneumoconiosis of China (GBZ70-2002)" and occupational acute allergic alveolitis according to "Diagnostic Criteria of Occupational Acute Allergic Alveolitis of China (GBZ60-2002)".

Lung function tests: Workers work an 8-h shift each day, working 6 d and resting for 2 d. Spirometry was performed with a ST-300 Spiro analyzer (Fukuda Sangyo

Co, Ltd, Japan) before beginning work on the first day after resting for two days. The subjects performed the maximum expiratory flow volume curve and repeated the performance until at least three acceptable curves were obtained. Subjects who failed to produce acceptable curves were excluded. Analysis was performed on the curve with the highest value. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), forced expiratory flow rate between 25 to 75% of FVC (FEF_{25-75}), and forced expiratory flow at 50% vital capacity (FEF_{50}) were analyzed. Multiple regression equations were established on the base of lung function data of the group of control workers. The predicted lung function ($\%\text{FVC}$, $\%\text{FEV}_1$, $\%\text{FEF}_{25-75}$, $\%\text{FEF}_{50}$) values for each worker were calculated according to the established equations. The lower limits of abnormal values were 80% for predicted FVC or FEV_1 , and 70% for predicted FEF_{25-75} and FEF_{50} .

Specific IgE antibody to A. fumigatus and Rhizopus nigricans: Five milliliters of sera of exposed and control workers were prepared and stored. AlaSTAT®EIA Allergen-Specific IgE tests for A. fumigatus and Rhizopus nigricans from DPC (Diagnostic Product Corp. Los Angeles, USA) were utilized (see Appendix II). DPC's SPHEIA 2000 spectrophotometers were programmed to reduce AlaSTAT EIA Allergen-Specific IgE data. The IgE concentrations of calibrators, controls, and samples are automatically calculated and printed out, along with a class number for each allergen tested. The standards for specific IgE antibody were: Specific IgE=0, can't be tested; 0 degree, 0.00–0.29 IU/ml; 0/I degree, 0.30–0.34 IU/ml; I degree, 0.35–1.49 IU/ml; II degree, 1.50–2.99 IU/ml; III degree, 3.00–14.99 IU/ml; IV degree, more than 15.00 IU/ml. DPC declare that the clinical specificity of the AlaSTAT methods is greater than 95%, and the sensitivity was found to be above 95%. High levels of inhibition proved that the antibody was specific for the allergen and in the allergen-inhibition tests, antibody specificity was above 90%.

Skin Prick Test (SPT): All 130 exposed workers were tested by the same physician by prick puncture using a standardized prickler. Commercially available allergens (A. fumigatus and Rhizopus nigricans from Diagnostic Product Corp. Los Angeles, USA) were used. In all workers we used the volar surfaces of both forearms. Allergen solutions were placed in the same site in order to obtain comparable SPT's results. Only reactions ≥ 3 mm diameter were considered positive. Reactions were read after 15 min by using a millimeter ruler. The wheal diameters were compared to the size of a positive histamine control (10 mg/ml) and to a negative control (solution without allergen). A wheal of the same size as that induced by the histamine control is indicated as +++

and a reaction similar to that induced by the negative control is indicated as -. The gradings + and ++ were used for reaction size between the negative and the positive controls. For a reaction larger than +++, a plus is added for each doubling of the average diameter.

Statistical analysis: Differences in the numbers of fungi colonies were analysed by Poisson's distribution test. Differences in the prevalences of respiratory symptoms, prevalences of abnormal lung function and the antibody levels to *A. fumigatus* and *Rhizopus nigricans* were examined by the chi-square test. Differences were considered as significant at $p < 0.05$.

Results

Totally, 121 samples of the dust were collected in the tobacco processing workshops. The average dust concentrations ranged from 13.76 to 29.55 mg/m³, which were above the national health limit of China, 3 mg/m³. Silica content ranged from 2.57% to 11.62% (Table 1). Sandy soil was the source of the silica in the tobacco dust, and it was attached to the tobacco leaves. The sizes of dust particles in the different tobacco processing workshops were similar: 62–75% of the particles were under 5 µm; there were more than 36% of particles under 3 µm. This indicates that most of tobacco dust in this

factory was respirable.

The data of isolated fungi in tobacco processing workshops in different months compared with the data in control environments of the machine factory are presented in Table 2. The numbers of fungi colonies in various workshops were similar in different months. Most of the total numbers of fungi colonies in the tobacco processing workshops were significantly higher than those of the control environments ($p < 0.05$ or $p < 0.01$).

The distribution of atmospheric fungi in various tobacco processing workshops is shown in Table 3. The fungal spectrum among all workshops was almost similar. *Rhizopus nigricans*, *A. fumigatus*, *Penicillium*, *Aspedillum ferreus* and others in the tobacco processing workshops were significantly higher than those in the control environments ($p < 0.01$). Nevertheless, the leading strains in the control environments were *Alternaria alternata* and *Cladosporium cladosporioides*.

The prevalences of respiratory or nasal symptoms (chronic cough, chronic phlegm, dyspnea, chest tightness, snuffle, snivel and allergic rhinitis) in exposed groups were higher than those of control groups (Table 4). The prevalences of the symptoms in exposed male workers were 32.86% with chronic cough, 28.57% with chronic phlegm, 24.29% with dyspnea, 20.00% with chest tightness, 25.71% with snuffle, 30.00% with snivel, which

Table 1. Dust concentrations and silica contents in workshops

Workshops	Samples	Dust concentration (mg/m ³)			Silica content (%)
		Maximum	Minimum	Means (TWAs)	
Unpacking	25	45.6	8.7	26.53	10.23
Baking	25	58.3	7.4	20.09	8.37
Reeling off	25	51.8	3.9	29.55	11.62
Blending	25	33.0	5.1	16.82	3.69
Wrapping	21	36.6	2.8	13.76	2.57

Table 2. The total numbers and relative numbers of isolated fungi colonies in the workshops in different months

	Unpacking	Baking	Reeling off	Blending	Wrapping	Control
April	117** (3.25)	102** (3.33)	84** (2.33)	67** (1.86)	31 (0.86)	36
August	155** (3.78)	125** (3.05)	88** (2.15)	34 (0.83)	63* (1.54)	41
December	149** (9.93)	112** (7.47)	93** (6.20)	50** (3.33)	42** (2.87)	15
Total	421** (4.58)	339** (3.68)	265** (2.88)	151** (1.64)	137** (1.49)	92

Data in brackets are the ratios of exposed groups to control ones. Compared with control environment, * $p < 0.05$, ** $p < 0.01$.

Table 3. Distribution of fungal spectrum in workshops

	Unpacking	Baking	Reeling off	Blending	Wrapping	Control
Rhizopus nigricans	52* (6.50)	61* (7.63)	40* (5.00)	32* (4.00)	26* (3.25)	8
A. fumigatus	15* (-)	19* (-)	13* (-)	8* (-)	6* (-)	-
Penicillium	66* (4.40)	32* (2.13)	44* (2.93)	15 (1.00)	17 (1.13)	15
Aspedillum ferreus	29* (-)	19* (-)	13* (-)	4* (-)	6* (-)	-
Sormodendrum	9* (-)	6* (-)	7* (-)	4* (-)	3* (-)	-
Monosporium	12* (-)	8* (-)	6* (-)	2* (-)	3* (-)	-
Aspdeillum versicolor	97* (16.17)	62* (10.33)	36* (6.00)	31* (5.17)	26* (4.33)	6
Aspedillum flavus	6* (6.00)	7* (7.00)	4* (4.00)	5* (5.00)	2 (2.00)	1
A. niger	57* (14.25)	43* (0.75)	34* (8.50)	23* (5.75)	9* (2.25)	4
Chaetomium SP	29* (-)	27* (-)	21* (-)	15* (-)	19* (-)	-
Cladosporium cladostorioides	8 (0.36)	6 (0.21)	4 (0.18)	3 (0.14)	3 (0.14)	22
Alternaria alternata	- (-)	- (-)	- (-)	- (-)	- (-)	36
Others	41* (-)	49* (-)	43* (-)	9* (-)	17* (-)	-
Total	421* (4.58)	339* (3.68)	265* (2.88)	151* (1.64)	137* (1.49)	92

Data in the brackets are the ratios of exposed groups to control ones. Compared with control environment, * $p < 0.01$

Table 4. The prevalences of respiratory symptoms in exposed and control groups (%)

Symptoms	Exposed group		Control group	
	Male (70)	Female (60)	Male (42)	Female (70)
Chronic cough	32.86**	28.33**	7.14	8.57
Chronic phlegm	28.57**	21.67	4.76	11.43
Dyspnea	24.29**	18.33	2.38	7.14
Chest tightness	20.00*	16.67**	4.76	2.86
Snuffle	25.71*	26.67*	9.52	10.00
Snivel	30.00**	25.00*	7.54	8.57
Allergic rhinitis	11.43	11.67	0	0

Snuffle : the act of breathing heavily through the nose when the nose is congested. Snivel: the act of snuffing up mucus through the nose.

Compared with control groups, * $p < 0.05$, ** $p < 0.01$.

Table 5. The prevalences of abnormal lung function in exposed and control groups (%)

Lung function	Exposed group		Control group	
	Male (70)	Female (60)	Male (42)	Female (70)
FVC	17.14*	41.60**	2.38	10.94
FEV ₁	22.86*	30.00*	7.14	14.06
FEF ₂₅₋₇₅	34.29*	28.33	16.67	15.63
FEF ₅₀	28.57	25.00*	19.05	9.38

Compared with control groups, * $p < 0.05$, ** $p < 0.01$.

Table 6. Specific IgE levels to *A. fumigatus* and *Rhizopus nigricans*

Degree	<i>A. fumigatus</i>		<i>Rhizopus nigricans</i>	
	n	Prevalence (%)	n	Prevalence (%)
0	80	61.54	56	43.08
0/I	15	11.54	7	5.38
I	9	6.92	58	44.62
II	8	6.15	4	3.08
III	6	4.62	3	2.31
IV	12	9.23	2	1.54

I–IV degree (35 positive, 26.92%) of specific IgE to *A. fumigatus* in exposed group compared with that (no positive) of control group, $p < 0.01$. I–IV degree (67 positive, 51.54%) of specific IgE to *Rhizopus nigricans* in exposed group compared with that (1 positive, 0.89%) of control group, $p < 0.01$.

Table 7. Skin prick test of exposed workers

Test	Degree	<i>A. fumigatus</i>		<i>Rhizopus nigricans</i>	
		n	Prevalences (%)	n	Prevalences (%)
Skin prick test	–	106	81.54	99	76.15
	+	9	6.92	16	12.31
	++	14	10.77	13	10.00
	+++	1	0.77	2	1.54

were significantly higher than those in male control workers ($p < 0.05$ or $p < 0.01$). The prevalences of the symptoms in exposed female workers were 28.33% with chronic cough, and 16.67% with chest tightness, 26.67% with snuffle, 25.00% with snivel, which were significantly higher than those in female control workers ($p < 0.05$ or $p < 0.01$). Although no cases were able to be diagnosed as pneumoconiosis or occupational acute allergic alveolitis, 10 male cases (14.29%) and 4 female cases (6.67%) had X-ray film findings of severe enhanced lung markings, and 4 male cases showed spotted shadows.

The abnormalities in lung function in the exposed and control groups are shown in Table 5. The prevalences of lung function abnormalities in exposed male workers

were 17.14% with FVC, 22.86% with FEV₁, 34.29% with FEF₂₅₋₇₅, which were significantly higher than those in male control workers ($p < 0.05$). The prevalences of lung function in exposed female workers were 41.60% with FVC, 30.00% with FEV₁, 25.00% with FEF₅₀, which were significantly higher than those in female control workers ($p < 0.05$).

Table 6 shows specific IgE antibody levels to *A. fumigatus* and *Rhizopus nigricans*. Among 112 control workers, 111 workers showed 0 degree of specific IgE to *A. fumigatus* and 1 worker showed 0/I degree; 106 control workers showed 0 degree of specific IgE to *Rhizopus nigricans*, 5 workers showed 0/I degree, and only 1 worker showed I degree (positive). Among the

130 exposed workers, 35 (26.92%) or 67 (51.54%) workers showed I–IV degree of specific IgE to *A. fumigatus* and *Rhizopus nigricans*, respectively, significantly higher numbers than those in control workers ($p < 0.01$). The results of the skin prick test for 130 exposed workers are shown in Table 7. Twenty-four (18.46%) workers were positive to *A. fumigatus* and 31 (23.85%) were positive to *Rhizopus nigricans*.

Discussion

Tobacco dust with complex components may cause respiratory disease, but the etiologic agents remained unresolved. Respiratory or nasal symptoms in cigar and cigarette making workers were significantly higher than controls¹. Occupational asthma and chronic bronchitis were found in tobacco workers². Also, tobacco workers tend to have lower lung function^{3,4}. Our investigation showed that the average dust concentrations ranged from 13.76 to 29.55 mg/m³, which were much higher than the national health limit of China, 3 mg/m³. The prevalences of respiratory symptoms in exposed groups were significantly higher than those of the control group. The prevalences of lung function abnormalities in exposed workers were significantly higher than those in control workers. We also demonstrated that there is an increased risk of respiratory symptoms and lung function abnormalities as a result of high concentrations of tobacco dust in the tobacco processing factory.

In some tobacco factories, tobacco processing workshops are moist and have no ventilation system. In this kind of condition, the concentrations of fungi may be higher. Fungi and its spores, mycelium and metabolic products might be allergenic agents^{6,8,9}. Allergy to tobacco products may be an occupational hazard and allergic reactions to tobacco antigens in tobacco leaves might be responsible⁵. Exposure to spores of different molds in the manufacture of tobacco products may induce symptoms and signs relating to extrinsic allergic alveolitis⁶. We observed that the numbers of fungi colonies in the tobacco processing workshops were much higher than those of the control environments. *Rhizopus nigricans* and *A. fumigatus* in the tobacco processing workshops were the leading strains. The prevalences of chronic respiratory or nasal symptoms including snuffle, snivel and allergic rhinitis in exposed groups were significantly higher than those in control workers. Although no case was diagnosed as pneumoconiosis or occupational acute allergic alveolitis, 14 cases had X-ray film findings of severe enhanced lung markings and 4 male cases showed spotted shadows. Specific IgE antibody levels to *A. fumigatus* and *Rhizopus nigricans* showed that 26.92% or 51.54% of the exposed workers were positive respectively, significantly higher percentages than those of control workers. The results of the skin prick test of the exposed workers indicated

that 18.46% of the workers were positive to *A. fumigatus* and 23.85% were positive to *Rhizopus nigricans*. Based on our preliminary study, we considered that microorganisms, especially fungi, might be one of the allergens causing allergic respiratory or nasal diseases among the tobacco processing workers.

Appendix I: The respiratory symptom questionnaire

- (1) Cough: ①Do you usually cough first thing in the morning (on getting up) in the winter? and/or ②Do you usually cough during the day-or at night-in the winter? ③Do you usually cough like this most days (or nights) for as much as three months each year? Phlegm: ①Do you usually bring up any phlegm from your chest first thing in the morning (on getting up) in the winter? and/or ②Do you usually bring up any phlegm from your chest during the day-or at night-in the winter? ③Do you usually bring up any phlegm from your chest like this most days (or nights) for as much as three months each year? Subjects were considered to have chronic respiratory symptoms if they had cough or phlegm production on most days or nights for as much as 3 months each year (chronic cough or chronic phlegm).
- (2) Dyspnea (shortness of breath): ①Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? ②Do you get shortness of breath walking with other people of your own age on level ground? ③Do you have to stop for breath when walking at your own pace on level ground?
- (3) Chest tightness: Do you get chest tightness if you are exposed to tobacco dust?
- (4) Sneez, or snuffle, or snivel: Do you have sneeze, or snuffle, or snivel if you are exposed to tobacco dust? The workers were considered to suffer from allergic rhinitis if they had sneeze, snuffle, and snivel occurrence more than half an hour each day for as much as 6 months each year.

Appendix II: AlaSTAT®EIA Allergen-Specific IgE tests

- (1) Add 50 μ l of each of the calibrators 0, A, B,C and D into the tube prepared.
- (2) Pipette 50 μ l of serum of each control and sample into the tube prepared for it.
- (3) Pipette 100 μ l of the selected Specific Allergen into each sample and control tube prepared for it.
- (4) Pipette 100 μ l of the Reference Allergen into each of the tubes labeled 0, A, B,C and D.
- (5) Shake for 1 h at room temperature (15–28°C) on a rack shaker set at approximately 200 strokes per minute.
- (6) Add 50 μ l of Anti-Ligand to all tubes except the BLK tubes.
- (7) Shake for 1 h at room temperature on a rack shaker set at approximately 200 strokes per minute.

- (8) Decant the contents of every tube. Invert the tubes on absorbant paper and remove as much moisture as possible. Add 2.0 ml Buffered Wash Solution, let the wash sit in the tubes for 2 min. Decant and again remove as much moisture as possible by tapping the tubes on fresh layers of absorbant paper. Repeat this process once more, for a total of 2 washes.
- (9) Add 200 μ l of Enzyme-Labeled Anti-IgE Ab to all tubes, except the BLK tubes.
- (10) Shake for 1 hour at room temperature on a rack shaker set at approximately 200 strokes per minute.
- (11) Decant and wash all tubes 3 times. After the last wash, allow the tubes to drain for 2 or 3 min, then strike the tubes sharply on fresh layers of absorbant paper to shake off all residual droplets.
- (12) Prepare the Substrate Working Solution by combining. For each 5 ml required: 1 OPD Tablet with 5 mL Buffered Peroxide Solution is required.
- (13) Add 500 μ l of Substrate Working Solution (freshly prepared) to all tubes, including the BLK tubes.
- (14) Incubate all tubes for exactly 15 min at room temperature on a rack shaker.
- (15) Add 500 μ l of 1 N sulfuric acid to all tubes including the BLK tubes. Shake the rack.
- (16) At 490 nm, using a spectrophotometer set to zero with the BLK (substrate blank) tubes, read the absorbance of each tube.

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