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Field Study

Environmental Mycological Study and Respiratory Disease Investigation in Tussah Silk Processing Workers

Jie CHEN, Jin SHI, Shusen WANG, Sufen YANG, Jiezhi LOU and Zhenlin LIU

Division of Pneumoconiosis, School of Public Health, China Medical University, P. R. China

Abstract: Environmental Mycological Study and Respiratory Disease Investigation in Tussah Silk Processing Workers: Jie CHEN, *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, pulmonary function and chest X-ray examinations, and analysis of antibodies to fungi of 197 tussah silk-processing workers and 40 control workers. An industrial hygiene survey and environmental mycological studies were also conducted. The dust concentrations in tussah silk processing workshops were less than 5.1 mg/m^3 on average, with a maximum of 7.8 mg/m^3 below the national health limit of 10 mg/m^3. Most dusts in all tussah silk processing workshops contained less than 1.2% silica. Numbers of isolated fungi in tussah silk processing workshops [755–6,544 cfu/m^3 (colony forming unit/m^3), were significantly higher than those in control environments (63–472 cfu/m^3). The prevalences of respiratory symptoms in tussah silk processing workers were higher than those in control workers. The prevalences of respiratory symptoms in exposed male non-smoking workers were 44.4% with chronic cough, and 38.9% with chronic phlegm respectively, which were significantly higher than those (12.5%, 12.5% respectively) in male non-smoking control workers (p<0.05). The prevalences in exposed male smoking workers were 42.9% with dyspnea, and 38.1% with chest tightness respectively, which were significantly higher than those (16.7%, 8.3% respectively) in male smoking control workers (p<0.01). The prevalences of respiratory symptoms in exposed female workers were 25.3% with chronic cough, 38.0% with chronic phlegm, 31.0% with dyspnea, and 29.1% with chest tightness respectively, which were significantly higher than those (10.0%, 10.0%, 10.0%, 5.0% respectively) in female control workers (p<0.01). Fifteen exposed workers often suffered from fever. Five X-rays were abnormal and four cases had nodular or patchy shadows. The prevalences of pulmonary function abnormalities in the exposed female group were significantly higher than those in control groups (p<0.01). The OD_{450nm} values for antibodies to fungi in tussah silk processing workers were significantly higher than those of control workers (p<0.05). The positive rates of anti-fungal antibodies in tussah silk-processing workers were also significantly higher than those of control workers (p<0.01). The results suggested that fungi might be one of the main allergens in respiratory diseases in the tussah silk processing workers.
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Correspondence to: J Chen, Division of Pneumoconiosis, School of Public Health, China Medical University, 92 North 2nd Road, Shenyang, 110001, P. R. China
(e-mail: chenjie@mail.cmu.edu.cn)

In China, the tussah silk-processing industry is mainly located in Liaoning province, in the northeastern part of China. The components of tussah silk dust are very complex. There might be a correlation between tussah silk dust and respiratory disease, mainly respiratory allergic diseases[1]. We conducted an investigation of respiratory symptoms, chest X-ray examinations, pulmonary function and analysis of antibodies to fungi of 197 tussah silk-processing workers and 40 control workers. An industrial hygiene survey and environmental mycological studies were also conducted. We also discussed possible causes of respiratory injury in this tussah silk-processing factory.

Materials and Methods

Subjects: The tussah silk-processing factory studied was founded in 1993. The primary raw materials (cocoons) were processed in the following steps: selecting, washing, roving, spinning, copping, and winding.

A total of 197 workers in the tussah silk-processing factory were investigated in this study. The three criteria for subject-selection were (1) exposure to tussah silk dust; (2) no other exposure to toxicants; (3) absence of tuberculosis or heart disease. Among them, 21 male

smoking workers were 25.1 ± 7.5 yr old on average (18–46 yr old) and the average work duration was 4.3 ± 2.5 yr, and 18 male non-smokers were 21.6 ± 5.3 yr old on average (17–41 yr old) and average work duration was 3.4 ± 2.2 yr, and 158 female workers (non-smokers) were 19.0 ± 4.0 yr old on average (17–34 yr old) and the average work duration was 1.8 ± 1.4 yr. Another 40 workers with similar labor intensity and no exposure to dust or toxicant in an appliance assembling factory were selected as controls. 12 male smokers were 31.8 ± 8.8 yr old on average (19–47 yr old); 8 male non-smokers 32.3 ± 9.7 yr old on average (19–45 yr old); 20 females (non-smokers) 30.1 ± 8.3 yr old on average (18–44 yr old).

Environmental surveys of dust concentration, free silica content and environmental mycological study: Workers work an 8-h shift each day, working 6 days and resting for 2 days. The total dust concentration was measured by area sampling, and 2–4 locations for each tussah silk processing workshop were selected for sampling. Four samples were collected during the entire 8-h shift, and sampling pumps were utilized. The arithmetic means of dust concentrations in all workshops were calculated as the time-weighted average (TWAs) according to the data obtained in the surveys. Size distribution of the dust was examined with a microscope. The gravimetric method was carried out to analyze the silica content. Out-plate culture (90 mm) was adopted to inspect the fungus conditions in all workshops in the tussah silk-processing factory and in the control workshops in the appliance assembling factory. The culture plates used for sampling atmospheric molds contained Sabouraud's agar medium with gentamicin (50 μg/m*l*). Five plates were exposed for 5 min in the workers' breathing zone (1–1.5 m high) for each processing workshop. Their 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 and smoking habits were collected by means of a self-administered Chinese version of the British Medical Research Council (BMRC) Standardized Questionnaire. Additional questions about work-related symptoms, fever and occupational history were included. Questions on Cough and Phlegm were as follows. 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 on most days (or nights) for as much as three months each year? Phlegm: ①Do you usually bring up phlegm from your chest first thing in the morning (on getting up) in the winter? and/or ②Do you usually bring up 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 on most days (or nights) for as much as three months each year? Subjects were considered to have chronic respiratory symptoms if they had a cough or phlegm production on most days or nights for as much as 3 months each year (chronic cough or chronic phlegm). Dyspnea (shortness of breath) was defined if they answered yes to one of the following three questions. 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 ? Chest tightness or fever was considered to be positive if they answered yes to each of the following questions: "Do you usually get chest tightness if you are exposed to tussah silk dust?" or "Do you usually have fever after being exposed to tussah silk dust?" Chest X-ray films were taken for those with at least 3 respiratory symptoms. Chest X-ray films were examined by a radiologist. The films with spotted or patchy shadows or intermediate or severe enhanced lung-marking were considered to be abnormal.

Pulmonary function tests: 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 produced 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), maximum midexpiatory flow (MMF), forced expiratory flow at 50% vital capacity (V_{50}) and forced expiratory flow at 25% vital capacity (V_{25}) were analyzed. Multiple regression equations had been established by our division[2] on the basis of pulmonary function data for a group of nonsmoking control workers. The predicted pulmonary function (%FVC, %FEV_1, %MMF, %V_{50}, %V_{25}) 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 V_{50}, MMF or V_{25}.

Specific IgG to fungi by ELISA (Enzyme-linked immunosorbent assay): 5 m*l* sera of 197 exposed workers and 40 control workers were prepared and stored at –20°C. 6 mg mycelia of fungi were homogenized completely into a carbonate buffer solution (PH 9.6) by ultrasonic treatment. The supernatant after centrifugation at 40,000 g for 10 min was taken as ELISA antigen solution. 100 μ*l* of antigen solution per well was added and preserved at 4°C for 24 h for antigen coating. The plates were washed 3 times with phosphate-buffered

saline (PBS). The 100 μl of 1:100 dilutions of serum was put into the antigen-coated well and cultured for 1 h at room temperature. After washing 3 times, 100 μl sheep anti-human IgG-HPR was added, then incubated at 37°C for 45 min, After being washed 3 times, to each well was added 100 μl TMB (H_2O_2/3,3',5,5'-tetramethyl-phenylamine), and left at 25°C for 15 min. The enzymatic reaction was stopped with 50 μl 2M sulphuric acids and the absorbances read at 450 nm with a spectrophotometer (Bio rad-450).

Statistical analysis: Differences in the proportion of respiratory symptoms, prevalence of abnormal pulmonary function and positive rates of anti-fungal antibodies were examined by chi-square test. Differences in OD_{450nm} values for specific antibodies were examined by Student's t test. $p<0.05$ was regarded as the level of significance.

Table 1. Dust concentrations in the workshops

Procedures	Dust concentration (mg/m^3)		
	Maximum	Minimum	Mean
Selecting	7.8	3.4	5.1
Washing	3.9	1.7	2.6
Roving	6.5	1.6	3.8
Spinning	6.4	1.3	3.3

Results

Most of the dust concentrations in tussah silk processing workshops were less than 4.0 mg/m^3 on average. The maximum was 7.8 mg/m^3, which was below the national health limit of China 10 mg/m^3 (Table 1). Most dust contained less than 1.2% silica, and silica content ranged from 0.6% to 2.7% silica. After averaging the results for dust size distribution from different workshops, we found that 66.4% of the particles were under 5 μm; 23.3% of the particles were between 5.0–10.0 μm; and only 10.3% of the particles were larger than 10 μm. This indicated that most dusts in this factory were respirable.

The data on isolated fungi in the tussah silk processing workshops compared with the data in control workshops in the appliance assembling factory are shown in Table 2. The numbers of fungi in the tussah silk processing

Table 2. Numbers of isolated atmospheric fungi (cfu/m^3) in each workshop

	Tussah silk processing factory									Appliance assembling factory	
	Selecting	Washing	Roving				Spinning	Copping	Winding	workshop I	workshop II
			Drawing	Carding	Spooling	Copping					
Sampling numbers	5	3	7	3	5	4	5	4	3	5	5
Fungal numbers	5,028 ± 140	3,665 ± 364	6,386 ± 349	4,404 ± 295	6,544 ± 319	5,820 ± 316	2,202 ± 203	755 ± 49	787 ± 69	63 ± 5	472 ± 44

The temperatures in all workshops were 26–29°C. Relative humidity was 52–81%.

Table 3. Fungal spectrum in the workshops

Workshops		Cladosporium	Penicillium	Alternaria	Aspergillum	Yeast	Others	Total colonies
Selecting		15 (9.4)	3 (39.6)	38 (23.9)	23 (14.5)	10 (6.3)	10 (6.3)	159
Washing		24 (34.3)	20 (28.6)	18 (25.7)	5 (7.1)	3 (4.3)	–	70
Roving	Drawing	98 (34.5)	96 (33.8)	37 (13.0)	13 (4.6)	19 (6.7)	21 (7.4)	284
	Carding	54 (64.3)	12 (14.3)	4 (4.8)	10 (11.9)	4 (4.8)	–	84
	Spooling	70 (33.7)	50 (24.0)	56 (26.9)	13 (6.3)	10 (4.8)	9 (4.3)	208
	Copping	64 (43.2)	40 (27.0)	24 (16.2)	16 (10.8)	–	4 (2.7)	148
Spinning		18 (25.7)	24 (34.3)	14 (20.0)	–	9 (12.9)	5 (7.1)	70
Copping		10 (52.6)	5 (26.3)	4 (21.1)	–	–	–	19
Winding		9 (60.0)	–	6 (40.0)	–	–	–	15
Others		81 (38.0)	63 (29.6)	43 (20.2)	17 (8.0)	3 (1.4)	6 (2.8)	213
Total		433 (34.1)	378 (29.8)	245 (19.3)	101 (8.0)	58 (4.6)	55 (4.3)	1270

Note: Data in parentheses are percentages of the fungus colony.

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

Symptoms	Exposed group			Control group		
	Male smokers (21)	Male non-smokers (18)	Female (158)	Male smokers (12)	Male non-smokers (8)	Female (20)
Chronic cough	42.9	44.4*	25.3**	25.0	12.5	10.0
Chronic phlegm	28.6	38.9*	38.0**	25.0	12.5	10.0
Chest tightness	38.1**	16.7	29.1**	8.3	12.5	5.0
Dyspnea	42.9**	16.7	31.0**	16.7	12.5	10.0
Fever	–	–	9.5**	–	–	–

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

Table 5. The prevalences of abnormal pulmonary function in exposed and control groups

Index of Pulmonary Function	Exposed group			Control group		
	Male smokers (21)	Male non-smokers (18)	Female (158)	Male smokers (12)	Male non-smokers (8)	Female (20)
FVC	9.5	16.7	38.0**	8.3	12.5	10.0
FEV_1	4.8	5.6	34.8**	8.3	12.5	10.0
MMF	4.8	27.8	46.8**	16.7	12.5	10.0
V_{50}	19.0	16.7	43.0**	16.7	12.5	15.0
V_{25}	9.5	5.6	28.5**	16.7	12.5	15.0

Compared with control groups, **: $p<0.01$

workshops were 755–6,544 cfu/m^3 (colony forming unit/m^3). The numbers of fungi in the control environments of the appliance assembling factory were much lower (63–472 cfu/m^3).

The details of distribution of atmospheric fungi in various tussah silk processing workshops are shown in Table 3. A total of 1,270 fungal colonies were isolated from all the tussah silk processing workshops, and the fungal spectrum for all workshops was almost the same. The proportions of Cladosporium, Penicillium and Alternaria in all the colonies were 433/1,270 (34.1%), 378/1,270 (29.8%) and 245/1,270 (19.3%), respectively, but only 33 fungal colonies were isolated in the control environments of the appliance assembling factory. The leading strain in the control environments was Penicillium (13/33, 39.4%). Alternaria and Cladosporium were detected in only 7/33 (21.2%) and 4/33 (12.1%) of the colonies, respectively.

The prevalences of respiratory symptoms (chronic cough, chronic phlegm, dyspnea, chest tightness) and fever in exposed groups were higher than those in the control groups (Table 4). The prevalences of respiratory symptoms in exposed male non-smoking workers were 44.4% with chronic cough, and 38.9% with chronic phlegm respectively, which were significantly higher than those in male control workers ($p<0.05$). The prevalences of exposed male smoking workers were 42.9% with dyspnea and 38.1% with chest tightness respectively, which were significantly higher than those in male control workers ($p<0.01$). The prevalences of respiratory symptoms in exposed female workers were 25.3% with chronic cough, 38.0% with chronic phlegm, 31.0% with dyspnea, and 29.1% with chest tightness respectively, which were significantly higher than those in female control workers ($p<0.01$). Fifteen workers, all in the roving and spinning workshops, often suffered from fever, and complained of fever after exposure to dust for 3–5 h, and recovered by the next morning. In some cases, fever would last for 2–3 d. A total of 32 exposed workers who often had at least 3 types of symptoms were examined by chest X-ray. Five X-rays were abnormal. Among them, one had severe enhanced lung-marking; There were 4 with nodular or patchy shadows along the middle and lower fields of the lungs, in no more than 2 lung areas. Four control workers who often had at least 3 types of symptoms were examined by chest X-ray. Among them, none was abnormal.

The abnormalities in pulmonary function in exposed and control groups are shown in Table 5. Most of the prevalences of pulmonary function abnormalities in the exposed group were higher than those in the control groups. In exposed female workers, FVC, FEV_1, MMF,

Table 6. Mean absorbance in the ELISA test and positive rates of antibodies to fungi

Groups	No.	Cladosporium		Alternaria	
		OD_{450nm}	Positive rate (%)	OD_{450nm}	Positive rate (%)
Control	40	0.417 ± 0.116	2.5	0.493 ± 0.414	2.5
Dust-exposed	197	0.640 ± 0.288*	42.6**	0.778 ± 0.380*	45.2**

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

V_{50} and V_{25} abnormalities were significantly higher than those in the female control group ($p<0.01$).

Levels of antibody to fungi (Cladosporium and Alternaria) are shown in Table 6 as mean absorbance in an ELISA test (OD_{450nm}). The OD_{450nm} values for antibodies in 197 tussah silk processing workers were significantly higher than those in control workers ($p<0.05$). Based on the OD_{450nm} values for 40 control workers, 95 percent confidence intervals of OD_{450nm} values for anti fungi antibodies were calculated and the upper-limits of the 95 percent confidence intervals were established as the cut off values. The cut off values for specific antibodies to fungi were 0.644 for Cladosporium and 0.778 for Alternaria, respectively. The positive rates of antibodies to fungi in tussah silk processing workers were significantly higher than those of the control group ($p<0.01$, Table 6).

Discussion

Organic dust with complex components can cause respiratory diseases such as chronic bronchitis, exogenous allergic alveolitis (EAA), bronchial asthma and organic dust toxic syndrome (ODTS), but the etiologic agents remain unclear. Tussah silk is a type of animal organic dust. In the tussah silk processing environment, the main inhalation causative agents might be tussah silk dust itself, bacteria, endotoxin and fungi. Fungal spores, mycelium and metabolic products were all allergenic substances. It was reported that there were more than 10 species of fungi leading to EAA or bronchial asthma, such as Aspergillus fumigatus causing EAA and bronchial asthma, Alternaria inducing woodworker's lung and so on[1, 3–6].

In this tussah silk processing factory, most of the dust concentrations in the tussah silk processing workshops were less than 4.0 mg/m^3 on average, which were below the national health limit of China (10 mg/m^3). Most dust contained less than 1.2% silica, but the amounts of fungi in the tussah silk processing workshops were 755–6,544 cfu/m^3, and our findings of specific antibodies to fungi indicated that the tussah silk processing workers were exposed to fungi. The prevalences of respiratory symptoms (chronic cough, chronic phlegm, dyspnea, chest tightness) and fever in exposed workers were higher than those of control workers. Fifteen workers, all in the roving and spinning workshops often suffered from fever. Five X-ray films were abnormal, and 4 cases had nodular and patchy shadows. Based on our preliminary study, we consider that fungi might be one of the main etiologic agents causing the respiratory diseases in the tussah silk-processing workers.

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