

Expression of epithelial membrane antigen and cytokeratin among Indian workers exposed to cotton fibre dust in textile industries

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Abstract

Background: In India, around 20 million workers are engaged in the textile industries. However, the prevalence of byssinosis has been little reported.

Aims: To determine the prevalence of byssinosis and other respiratory disorders among workers exposed to cotton dust in textile mills in Delhi, India.

Methods: Sputum samples were collected from 156 workers employed in 15 cotton textile mills, and expression of epithelial membrane antigen (EMA) and cytokeratin (CK) marker proteins was investigated. Information regarding respiratory symptoms, certain personal characteristics and occupational history was also gathered.

Results: Symptoms were observed in 56.41% of the workers. Expression of EMA and CK was observed in 27.5% and 50% of the workers, respectively. Expression of EMA and CK was significantly associated with smoking and duration of employment.

Conclusion: Measures are needed to reduce dust levels in the workplace, and to discourage smoking and alcohol consumption among the textile workers.

Keywords: byssinosis, cotton dust, occupational health hazard, epithelial membrane antigen, cytokeratin, textile

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Introduction

People with mild byssinosis have a “Monday feeling” of chest tightness and shortness of breath on the first day of work after a weekend or holiday. As exposure continues, this feeling persists throughout the week, and in advanced stages, byssinosis causes chronic, irreversible obstructive lung disease. Although cotton is by far the most common cause – accounting for such conditions as cotton-dust asthma and cotton-mill fever – flax, hemp and other organic fibres can also produce byssinosis. In India, an estimated 20 million workers are occupationally exposed to cotton dust in textile manufacturing industries (1). Previous studies have assessed the association between the duration of employment and the emergence of respiratory symptoms, and have reported longitudinal changes in the pulmonary function test as annual decline in lung capacity, in addition to chest tightness, chronic bronchitis, and chronic cough, and sharp decline in forced expiratory volume in one second (2). Earlier studies have reported prevalence rates of byssinosis of approximately 30% in Indonesia, 37% in Sudan, 40% in Ethiopia, up to 50% in India, 18% in Cameroon, 14.2% in Turkey, 6.2% in France, 5.9% in Greece, 5% in Slovakia, and 1.7% in the Czech Republic (3–9). The present study was, therefore, aimed at reporting the prevalence of byssinosis and other respiratory disorders among workers exposed to cotton dust in textile mills situated in Delhi, India.

Methods

The study participants exposed to cotton dust in textile stitching units in Delhi, India, were selected using a simple random sampling technique. We enrolled 156 workers employed in these units, and their detailed history was recorded using a predesigned questionnaire, after which they provided sputum samples. The workers were asked to take a deep breath and cough hard to spit into a plastic cup that was later disposed according to the biomedical waste regulations. All procedures performed in the study involving human participants were in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Paraffin blocks of the sputum samples were made as described previously (10). Sections (5 µm thick) of the paraffin blocks were cut using a microtome, and stained with immunohistochemical molecular markers for epithelial membrane antigen (EMA) or cytokeratin (CK). Immunohistochemistry involved removal of the paraffin wax from the sections (10). Following this, antigen retrieval was performed using a microwave method (11). This used citrate buffer to expose the hidden antibody-binding sites. EMA marker protein (monoclonal antibody) was applied to the sections, and incubated overnight in a moist chamber at 4°C. On the next day, the sections were washed in phosphate-buffered saline (PBS), secondary antibody was applied for 20 minutes,

and the sections were kept at room temperature in a moist chamber. The sections were washed again in PBS, and tertiary antibody was applied for 20 minutes at room temperature in a moist chamber. This process increased the magnitude of the antibody–antigen reaction. The sections were washed one more time with PBS after 20 minutes. Diaminobenzidine was applied for staining of the cell nuclei. The same procedure was followed for measuring expression of cytokeratin (CK), by replacing EMA with CK, and using different sections of the same samples.

EpiData version 3.1 was used for quality data capture, and for univariate and bivariate analysis. The χ^2 test was used to measure the association between categorical variables. Multivariate logistic regression analysis of the different factors for EMA and CK was estimated, stratified by age, smoking or alcohol consumption status, duration of smoking or alcohol consumption, or employment. $P < 0.05$ was considered statistically significant.

Results

A total of 156 workers in 15 different textile stitching industries were surveyed: 150 men and 6 women; age range 16–60 years, median age 27 years. The mean duration of employment in the current section was 9.8 years, and the mean salary was INR 2881. The socioeconomic status of the workers was also recorded, and the number of workers earning (per month) in the range of INR 1000–2000, 2000–3000, 3000–4000 and 4000–5000 was 33, 74, 37 and 12, respectively.

The prevalence of respiratory disorders among workers was found to be 56.41% [95% confidence interval (CI): 45.24–69.50%]. All workers provided individual history and answered a detailed questionnaire focused on the diagnosis of respiratory disorders. The incidence was: chest pain (10.89%), chronic bronchitis (10.25%), tuberculosis (5.77%), back pain (5.12%), cough (4.48%), chronic productive cough (3.84%), insomnia (3.20%), and difficulty in breathing (1.28%) (Table 1).

Figure 1 shows the association between the number of smokers or drinkers and their economic status.

Table 1 Numbers of participants showing symptoms/diseases following working in the textile stitching industry ($N = 156$)

Symptoms/diseases	No. of individuals	Prevalence (%)
Chest pain	17	10.89
Cough	7	4.48
Chronic bronchitis	16	10.25
Chronic productive cough	6	3.84
Difficulty in breathing	2	1.28
Insomnia	5	3.20
Pain in back bone	8	5.12
Tuberculosis	9	5.77
Others	18	11.54

Figure 2 shows the duration of smoking or drinking. There was a significant association between smoking and economic status ($P < 0.01$). The overall incidence of symptoms/diseases in textile stitching workers who had never smoked was $< 5\%$. About 82% of the workers had never consumed alcohol. Most of the workers had been employed in the textile stitching industries for the last 5–10 years (Figure 3). The prevalence of symptoms such as chronic bronchitis, chest tightness, and chronic productive cough was more common among smokers and drinkers. There was a significant association between the duration of employment and prevalence of respiratory symptoms ($P < 0.05$).

Epithelial membrane antigen (EMA) expression was observed in 27.5% (95% CI: 19.48–37.87%) of the textile workers. The percentage of positive cells ranged from 40% to 50%, and the mean percentage positivity was 45% (Figure 4B). Logistic regression analysis revealed a significant association between EMA with smoking and its duration, alcohol consumption, and employment ($P = 0.0001, 0.02, 0.02, 0.0002$, respectively; Table 2). Expression of CK was observed in 50% (95% CI: 39.83–60.17%) of textile workers. The percentage of positive cells ranged from 38% to 60%, and the mean percentage positivity was 50.6% (Figure 4C). Logistic regression analysis revealed a significant association between CK and smoking and duration of employment ($P = 0.003$ and 0.0001 , respectively; Table 2).

Discussion

The findings of the present study are restricted to workers employed in textile stitching industries. There were fewer female workers and the age of the workers ranged from 16 to 60 years. Men within the 15–25 years age group were more sensitive to these respiratory symptoms. Smoking and alcohol consumption played a major role in causing symptoms/diseases including chronic bronchitis, chest tightness, cough, back pain, and breathing difficulty. The effect of this along with the textile environment has been reported to be additive (12). Most workers were from the Muslim community (82.05%), so habitual alcohol consumption was mostly absent, but those who consumed alcohol showed symptoms/diseases. It has been reported that with the increased duration of exposure, the prevalence of byssinosis also increases (5). Moreover, symptoms such as chronic bronchitis, chest tightness, and chronic productive cough have been observed to be more prevalent among smokers than to nonsmokers (13). Several studies have identified bacterial endotoxin in cotton dust as the main causative agent contributing to adverse respiratory effects (14,15). A 20-year follow-up cohort study performed on workers in cotton textile mills in Shanghai, China, showed that the chronic loss of lung function was significantly associated with exposure to endotoxin rather than cotton dust (2).

Proinflammatory cytokines act as stimuli for the activation of immune cells, as well as the release of further cytokines and chemokines, and their cellular

Figure 1 Association between number of workers with smoking or drinking habits and their monthly earning capacity

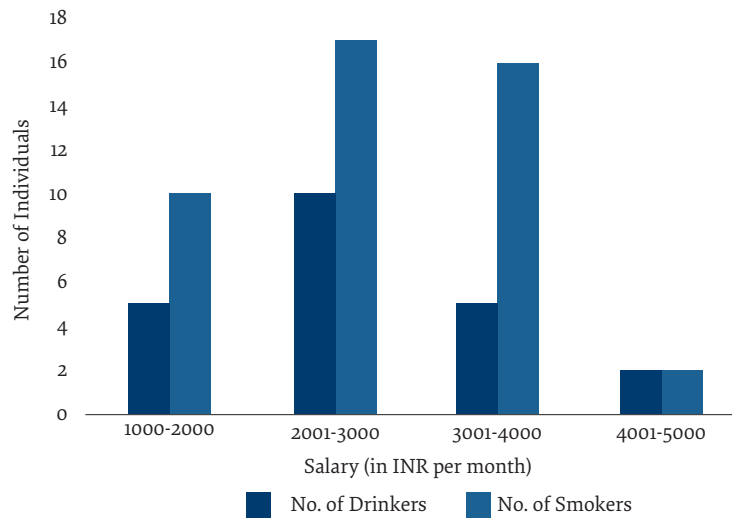


Figure 2 Duration of smoking or drinking habits of the workers

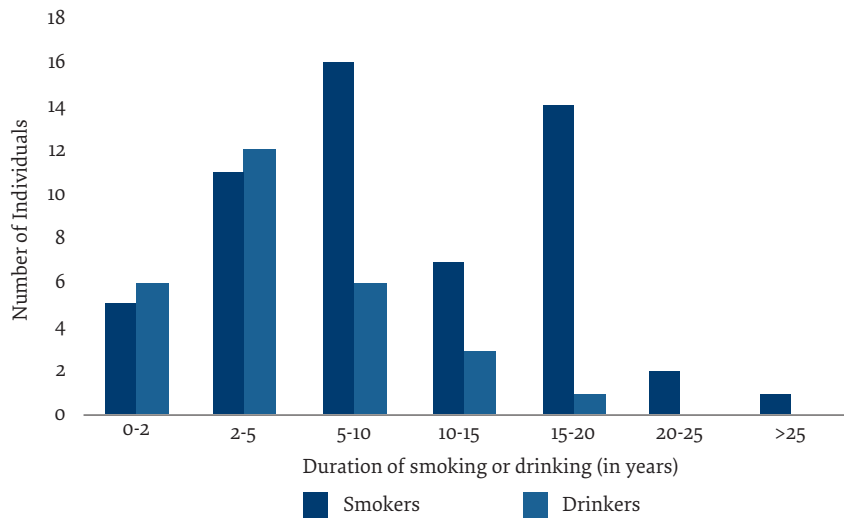


Figure 3 Duration of working of the individuals in the textile stitching industries

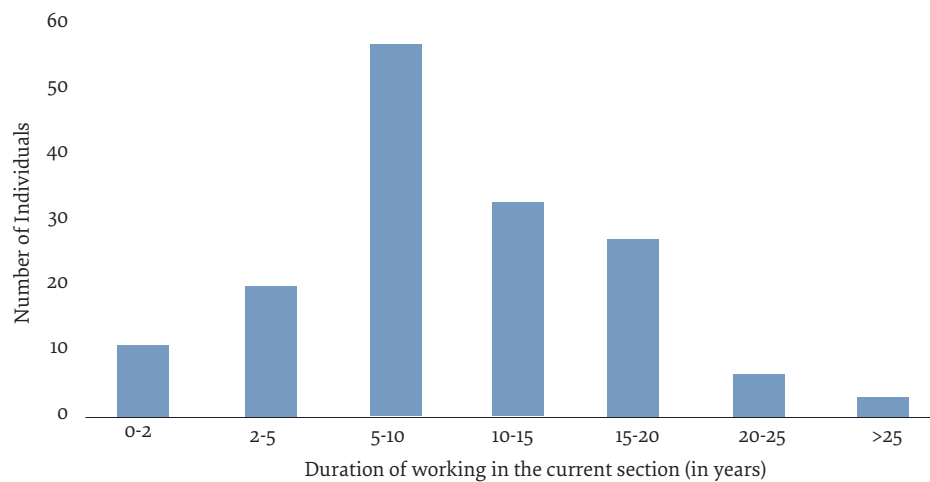
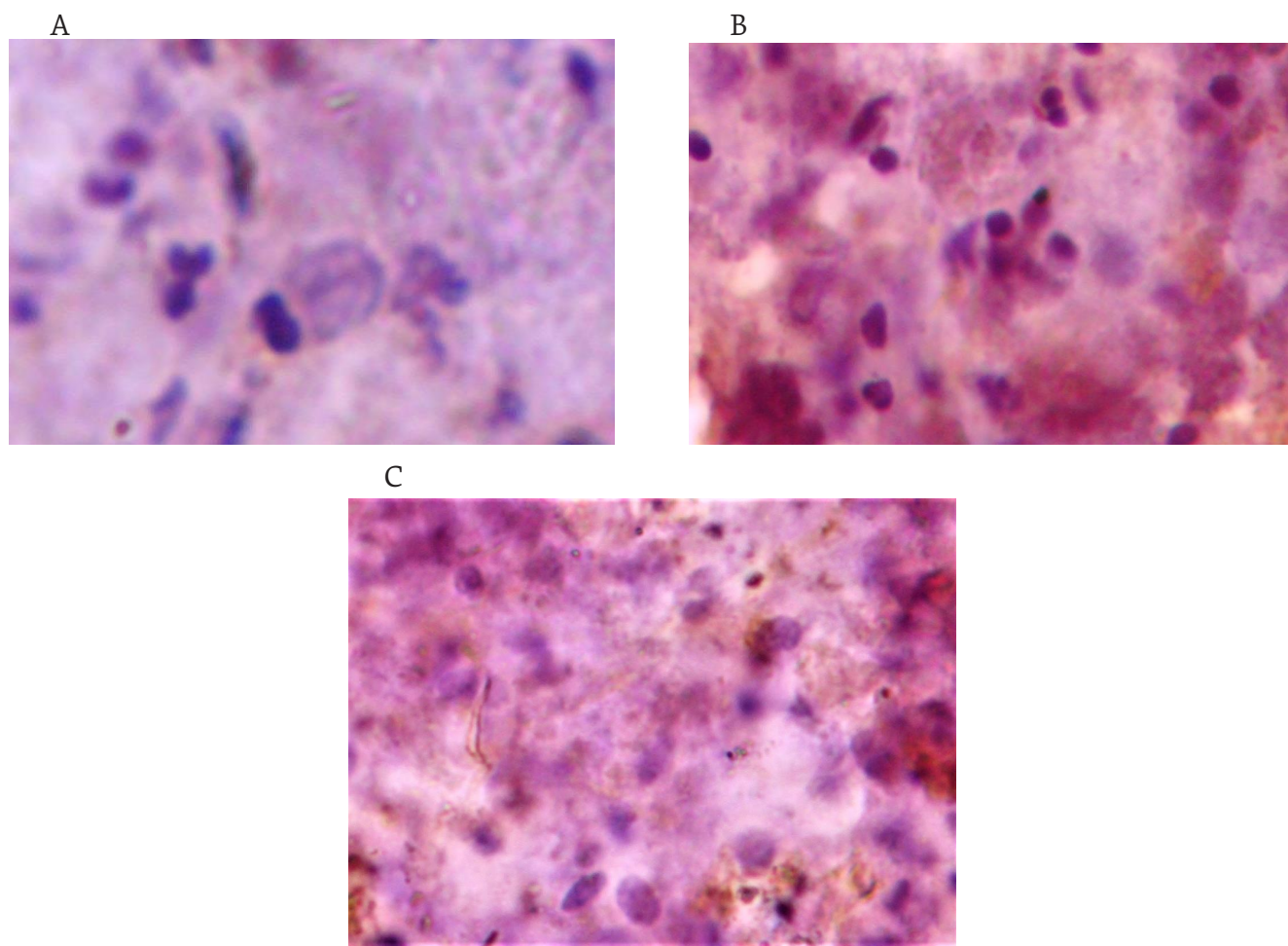


Figure 4 Expression of different immunological markers in sections of sputum blocks of (A) normal healthy, (B) EMA-positive and (C) CK-positive individuals



recruitment in bronchial epithelial cells (16). An earlier study has demonstrated that reactive oxygen species may have a role in the pathogenesis of airway inflammation and asthma (17). Eosinophils and neutrophils are characteristic features of asthma and allergic respiratory diseases (18). Acute exposure to cotton dust is reported to result in increased leukocyte count (19).

In the present study, immunohistochemistry was performed with EMA monoclonal antibody. EMA is useful for classifying tumours of epithelial origin and

is included in the group of antibodies directed against leukocyte common antigen. A similar pattern of staining is seen in other glandular epithelia, such as sweat glands, while squamous epithelium shows an uneven pattern of antigen expression. Expression of EMA was observed in 27.5% of textile workers. The percentage of positive cells ranged from 40% to 50%, and the mean percentage positivity was 45%.

CK is the major structural protein of bronchial epithelial cells. Several studies have suggested that CKs

Table 2 Multivariate logistic regression analysis of the different risk factors for EMA and CK (SA-156)

Variable	EMA OR (95% CI), P	CK OR (95% CI), P
Age	0.87 (0.45–1.67), 0.679	1.00 (0.63–1.61), 0.971
Smokers	15.59 (4.04–60.09), 0.0001	5.54 (1.73–17.67), 0.003
Alcohol consumption	5.50 (1.16–26.07), 0.031	1.84 (0.57–5.97), 0.304
Duration of smoking	6.44 (1.21–34.04), 0.028	4.14 (0.81–20.95), 0.085
Duration of alcohol consumption	3.51 (0.22–55.22), 0.371	3.23 (0.33–31.10), 0.309
Duration of employment	10.01 (2.94–34.0), 0.0002	9.53 (4.01–22.65), 0.0001

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are proteolysed during apoptosis and can leak into the circulation as a soluble form, where they may serve as new epitopes for antibody generation (20–22).

In the present study, 50% of cases were CK positive. CK is a cytoplasmic protein; therefore, monoclonal antibody binds to the antigen, leading to its expression. We ran a positive control and a normal control. The purpose of the positive control was to verify the staining procedure. CK expression in the normal control was 10%; however, in the workers, it ranged from 38 to 60%. Hence, it can be interpreted that cotton dust somehow influenced cell signalling, and this led to higher expression of CK.

Multivariate logistic regression analysis of the risk factors for EMA and CK revealed that their expression was associated with smoking, alcohol consumption, and duration of smoking or employment. Moreover, EMA and

CK expression was more significantly linked to smoking and duration of employment. Earlier studies have reported that smoking and duration of employment have a significant impact on workers employed in spinning mills, and coke oven workers exposed to polycyclic aromatic hydrocarbons (1,3,2,3,2,4).

In conclusion, the present study suggests the need for implementation of protective measures such as installation of hoods, dust filters, ventilators, general cleanliness, and other safety measures, such as the use of dust masks, to reduce the risks of respiratory problems. In addition, training on safety measures, health education and welfare programmes should be organized for textile industry workers.

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Competing interests: None declared.

Expression de l'antigène de la membrane épithéliale et de la cytokératine chez les travailleurs indiens exposés à la poussière de fibres de coton dans les industries textiles

Résumé

Contexte : En Inde, près de 20 millions de personnes travaillent dans les industries textiles. Cependant, la prévalence de la byssinose a été peu signalée.

Objectifs : Déterminer la prévalence de la byssinose et d'autres troubles respiratoires chez les travailleurs exposés à la poussière de coton dans les usines textiles de Delhi (Inde).

Méthodes : Des échantillons d'expectorations ont été recueillis auprès de 156 travailleurs dans 15 usines textiles de coton, et l'expression des protéines marqueurs de l'antigène de la membrane épithéliale et de la cytokératine a été étudiée. Des informations concernant les symptômes respiratoires, certaines caractéristiques personnelles et les antécédents professionnels ont également été collectées.

Résultats : Des symptômes ont été observés chez 56,41 % des travailleurs. L'expression de l'antigène de la membrane épithéliale et de la cytokératine a été observée chez 27,5 % et 50 % des travailleurs, respectivement. Celle-ci était fortement associée au tabagisme et à la durée d'emploi.

Conclusion : Des mesures sont nécessaires pour réduire les niveaux de poussière sur le lieu de travail, et afin de décourager le tabagisme et la consommation d'alcool parmi les travailleurs du textile.

الدليل على المستضدات الغشائية الظهارية والكيراتينات الخلوية بين العمال الهنود المعرضين لغبار الألياف القطنية في صناعات النسيج

نُرشل م بهتا ، سعود عزام ، أرشد رحمان ، مشاهد رزف ، نُرشل ما ندال

الخلاصة

الخلفية: مع جفاف الجلد عند نحو 70% من عمال مل فطمة ناعثا النسيج. ومع ذلك لم يتم الإبلاغ عن معدل انتشار السعال الحار القطن في أقاليم الهند إلا قليلاً.

الأهداف: هدفت هذه الدراسة إلى تحديد مدى انتشار السعال الحار القطن في أقاليم الهند، ونسبة الإصابة بالمرض بين عمال النسيج. هدفت هذه الدراسة إلى تحديد مدى انتشار السعال الحار القطن في أقاليم الهند، ونسبة الإصابة بالمرض بين عمال النسيج.

طرق البحث: تم إجراء بحث ميداني في مصنع نسيج الألياف القطنية، وتم فحص العمال على علا من البروتينات في المستضدات الغشائية الظهارية والكيراتينات الخلوية. كما تم إجراء فحص الدم لبعض العمال الذين يعانون من أعراض المرض.

النتائج: لوحظت أعراض المرض لدى 56,41% من العمال. ولوحظت دلالات على انتشار المرض في أقاليم الهند، ونسبة الإصابة بالمرض بين عمال النسيج. ولوحظت دلالات على انتشار المرض في أقاليم الهند، ونسبة الإصابة بالمرض بين عمال النسيج.

الاستنتاجات: يلزم اتخاذ تدابير للحد من مستويات الغبار في مكان العمل، ولتقليل التعرض عن التبغ والحد من استهلاك الكحول بين عمال النسيج.

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