Assessment of the impact of long exposure to cotton dust on respiratory health of workers in Minia city, Egypt

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Cotton dust, when breathed in, irritates the lungs and that may leads to byssinosis. To study the pulmonary functions among the exposed workers in different areas in spin factory in Minia city, Egypt and to identify the type and concentration of aerosol particles in the working areas as well as in the sputum culture. This cross sectional descriptive study was carried out on 128 workers who worked 5 years or more in the factory. Full history, clinical and radiological examinations, pulmonary function studies and sputum culture were done for all workers. Measurements of concentration of bacteria and fungi were carried out in two main working areas representing the high and the low exposure. Exposure to cotton dust for 5 years or more was associated with statistically significant increase in prevalence of chronic cough and dyspnea as well as decline in all pulmonary tests among high exposed workers compared to low exposed workers. Mean concentration of total collected fungi was 1215 cfu/m$^3$ at the high exposed area and 396 cfu/m$^3$ at the low exposed area. Aspergillus Nieger represented the most dominant fungal species at both areas. Gram –ve bacteria, klebsiela, was found in sputum culture of high exposed workers more than low exposed workers. Aspergillus Nieger represented the most fungal species in the sputum culture of high exposed workers, while among low exposed workers, it was mixed species.

Keywords: Spin factory, Cotton dust, Byssinosis, Pulmonary functions, respiratory symptoms, textile workers

INTRODUCTION

Cotton dust which is generated through the handling and processing of the cotton causes ill health to the workers. The dust is produced through the process of fabric production from the opening of cotton bolls in the field, harvesting and storing of seed cotton, separation of lint from seed, processing of yarn through to weaving or knitting into fabric (Furlow, 2011).

Cotton dust is a colorless, odorless solid. It may contain substances such as: non-cotton matter, bacteria, fungi, soil and pesticides which may have accumulated during the growing of the plant, harvesting of the crop and subsequent processing and storage periods. Breathing in cotton dust can cause serious, permanent lung damage (Paudyal et al., 2011; Dulkiewiez et al., 2001). Dust often acts as a carrier for biological particles whether naturally
occurring or artificially generated (Duquenne et al., 2013; Chanda, 1996).

Most airborne micro-organisms are in the fine dust fraction (particles size <7µm) and could easily deposit in the respiratory tract (Burge and Christine, 2000). Elevated levels of particle air pollution have been associated with decreased lung function, increased respiratory symptoms (such as cough, shortness of breath and wheezing), asthma as well as chronic obstructive pulmonary disease (COPD), cardiovascular diseases and lung cancer (WHO, 2002).

Diseases caused by inhalation of different bioaerosols depend not only on the biological properties and chemical composition of these bioaerosols but also on the number of particles inhaled and the site of their deposition in the respiratory system. The deposition site is directly related to the aerodynamic diameter of the particles therefore, the effect of these bioaerosols on human health depends significantly on their physical properties and size distribution. Particles larger than 10 µm have a low probability of entering and traveling the nasopharyngeal region of the respiratory tract, while bioaerosols of 5-10 µm diameters are mainly deposited in the upper respiratory tract and can cause, for example, allergic rhinitis. Particles smaller than 5 µm, called respirable fraction, are able to penetrate into lung alveoli causing allergic alveolitis and other serious illnesses (McIvor, 2012; Nagoda et al., 2012; Seltzer, 1992; Bakirci et al., 2006).

Inhalation of cotton, flax or hemp dust with the raw fibers, which often are contaminated with fungi and bacteria, may be responsible for acute reversible bronchoconstriction which on repeated exposure may lead to chronic disabling pulmonary disease, called byssinosis (Cui et al., 2011; Schilling et al., 1963).

Decline in forced expiratory volume in the first second (FEV1) has been noticed in cotton workers (Rajjer et al., 2013), although how far these are due to smoking and how far to cotton dust remains a matter of controversy. Bronchial reactivity is increased in most workers with byssinosis and fall in indices of small airway diseases have been taken to suggest that the physiological response may begin in peripheral airways (Fishwick et al., 1992). Early processes in textile factory (e.g. carding and blowing processes) are very dusty job and accompanied by more respiratory problems than spinning and twisting processes (Cullinan, 2012).

AIMS OF THE WORK

The current study aimed to: determine the effect of long term exposure to cotton dust on pulmonary functions and severity of respiratory diseases; identify the type and concentration of bacterial and fungal aerosol particles in the sputum of the workers as well as in the main working areas in the factory.

SUBJECTS AND METHODS

This cross sectional study was carried out from January 2013 to December 2013 on 128 workers in the cotton spin factory in Minia city, Egypt who worked more than 5 years in the factory. Each worker signed a written informed consent to take part in this research. The study was approved by the Ethical Committees of Minia Faculty of Medicine. Cullinan in 2012 suggested that carding and blowing processes are considered as high exposed processes to cotton dust while spinning and twisting processes are less exposed processes to cotton dust. Workers were divided into two groups:

High exposed group: included 40 workers from carding and blowing departments.

Low exposed group: included 88 workers from carding and blowing departments.

All participants were subjected to:

- Full medical history taking including smoking habit, respiratory symptoms (cough, expectoration, chest tightness) and occupational history including the usual daily hours of exposure to cotton dust and total duration of exposure in years.
- Chest radiography: both poster-anterior and lateral views were done.
- Pulmonary function tests using vitalograph – COMPACT: a portable spirometer that measures actual expiratory flow in addition to predicted values according to age, sex, height and race. The following parameters were measured and expressed as percentages.
  - FVC% = Forced vital capacity
  - FEV1% = Forced expiratory volume in the first second
  - FEV1/FVC% = Forced expiratory volume in the first second / forced vital capacity
  - PEFR % = Peak expiratory flow rate, and
  - FEF 25–75% = Forced expiratory flow at 25% to 75% of vital capacity (mid expiratory flow)

The volume-time curves (e.g. FVC, FEV1 and FEV1/FVC%) and the flow volume curves (e.g. PEFR and FEF 25–75%) were calculated according to acceptability and reproducibility criteria established by the American Thoracic Society (1987) (2 values of FEV1 and FVC should not differ by more than 5 % or 100 ml) (American Thoracic Society, 1987), the best of 3 acceptable curves was chosen. In normal young and middle-aged adults the FEV1/FVC% is usually above 75 %; while in the elderly values of 70-75 % are commonly found. Reduction in the FEV1/FVC% indicates airways narrowing, the severity of which is best measured by the absolute value of FEV1 (Morris et al., 1971).

Microbiological study

A. Environmental samples (Viable particles samples):

A six stage Andersen impactor (Andersen air sampler, Atlanta GA, USA) was used for collection and measurement of concentration of bacteria and fungi.
Table 1. The characteristic features of high exposed workers (carding and blowing department) and low exposed workers (twisting and spinning department)

<table>
<thead>
<tr>
<th>Variable</th>
<th>High exposed (n=40)</th>
<th>Low exposed (n=88)</th>
<th>t-test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47.0±5.8</td>
<td>46.3±5.9</td>
<td>0.75</td>
<td>0.41</td>
</tr>
<tr>
<td>Duration of employment/y</td>
<td>9.7 ± 3.2</td>
<td>10.9 ± 2.9</td>
<td>0.09</td>
<td>0.94</td>
</tr>
<tr>
<td>Working hours/day</td>
<td>6.1±0.3</td>
<td>6.2±0.25</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>Smokers</td>
<td>14 (35.0%)</td>
<td>22 (25.0%)</td>
<td>Z=0.91</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Table 2. Respiratory symptoms and x-ray findings among high and low exposed workers

<table>
<thead>
<tr>
<th>Variable</th>
<th>High exposed (n=40)</th>
<th>Low exposed (n=88)</th>
<th>z-test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic cough</td>
<td>38 (95.0%)</td>
<td>44 (56.8%)</td>
<td>13.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Expectoration</td>
<td>30 (75.0%)</td>
<td>34 (38.6%)</td>
<td>1.11</td>
<td>0.20</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>34 (85.0%)</td>
<td>55 (62.5%)</td>
<td>3.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Wheeze</td>
<td>28 (70.0%)</td>
<td>44 (50.0%)</td>
<td>2.6</td>
<td>0.08</td>
</tr>
<tr>
<td>X ray finding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>10 (25.0%)</td>
<td>41 (46.6%)</td>
<td>14.6</td>
<td>0.001</td>
</tr>
<tr>
<td>↑bronchovascular marking</td>
<td>12 (30.0%)</td>
<td>28 (31.8%)</td>
<td>0.05</td>
<td>0.40</td>
</tr>
<tr>
<td>Hyper inflation</td>
<td>18 (45.0%)</td>
<td>19 (21.6%)</td>
<td>0.05</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Sampling locations were chosen to represent the main working area; (1) Carding region: as a model for high dust concentration and (2) spinning region: represents the low dust concentration. The concentration of viable particles was estimated as colony forming unit per cubic meter of air (cfu/m³). Two different media were used for sample collection, Sabourauds Dextrose agar (SDA) supplemented with chloramphenicol for collecting fungal particles and blood agar for collecting bacterial particles. After 24-48 hours of incubation at 37°C, colonies on each plate were counted and identified.

B. Sputum samples collection and culture: Morning sputa were collected in sterile containers from all workers, processed and double inoculated on SDA (supplemented with chloramphenicol), MacConkey's agar, and blood agar media. All plates were incubated at 37°C and inspected daily for any significant growth (up to 2 weeks).

Identification of culture: Fungal isolates were identified macroscopically by colony morphology, texture, rising above surface and growth rate. Microscopic examination of stained smears from cultures may give an early indication. Gram's stain was used for bacterial films and lactophinol cotton blue for fungal smears.

STATISTICAL METHODOLOGY

Statistical analysis was carried out using SPSS Advanced Statistical Software version 19 (SPSS Inc., Chicago, USA). Quantitative data are presented as mean ± standard deviation while qualitative data are presented as frequencies and percentages. The unpaired student t test was used to compare between means. Test of proportion was used to compare between percentages. P values less than 0.05 were considered as statistically significant.

RESULTS

Table 1 showed the age of high exposed workers was similar to low exposed workers 47±5.8 vs 46.3±5.9 respectively. Working hours in the factory was from 9am to around 3pm therefore, the working hours were similar in both groups. All studied workers worked at least 5 years. High exposed workers worked for 9.7±3.2 years while low exposed workers worked for 10.9±2.9, but this difference was not statistically significant. Furthermore, number of smokers within the high exposed group was slightly more than that within the low exposed group (35% and 25% respectively, p=0.34).

Respiratory symptoms and chest x-ray of the workers were showed in table 2. It was found that 95% of high exposed workers suffered from chronic cough compared to 56.8% of the low exposed workers. This difference was statistically significant. Similarly, dyspnea was higher among high exposed than low exposed workers (85% and 62.5% respectively, p=0.04). On the other hand, expectoration and wheeze were slightly higher among high exposed than low exposed workers, but these differences were not statistically significant.

Chest x-ray was done for all workers. Only 25% of high exposed workers had free chest x-ray compared to 46.6% of low exposed workers (p<0.001). Increase bronchovascular marking and hyperinflation were found among 30% and 45% of high exposed workers, while these figures were 31.8% and 21.6% (respectively) among low exposed workers, but there were no statistical significant differences between both groups table 2.
The pulmonary function tests of the workers were shown in table 3. It was found that the mean values of FVC, FEV1, FEV1/FVC%, PEFR and FEF 25–75% among high exposed workers were lower than that among the low exposed workers, and these differences were highly statistically significant (p<0.001).

The mean concentrations of total collected fungi, individual fungal species and bacteria at both selected regions were represented in table 4. It was found that the mean concentration of total fungi was 1215 cfu/m$^3$, while that of Aspergillus niger was 993 cfu/m$^3$ at high exposed region which exceeded their values at low exposed region (total fungi was 396 cfu/m$^3$ and Aspergillus niger was 243.8 cfu/m$^3$). Moreover, Aspergillus niger represented the most dominant fungal species at both regions (81.7% and 61.6% of the total concentration at the high exposed and low exposed regions respectively, p<0.001). On the other hand the mean concentration of Penicillium was the lowest (88 cfu/m$^3$ in carding - blowing and 24.7 cfu/m$^3$ in spinning - twisting regions) and consequently showed the least percentages of the total concentration (7.3% at the high exposed region and 6.2% at the low exposed region, p=0.4). The results of Aspergillus flavus lie in the middle where its mean concentrations in both regions were 134 cfu/m$^3$ and 127.5 cfu/m$^3$ (11% and 32.2% respectively, p<0.001). A low concentration of bacteria was recorded at both regions (17.6 cfu/m$^3$ and 14.0 cfu/m$^3$ respectively) and they were of the gram negative species.

Table 5 showed the prevalence of bacteria and fungi in sputum samples of high and low exposed workers. Culture of sputum revealed that 22.5% of high exposed workers had Klebsiella growth, while 7% had growth at the low exposed region (z-test p=0.04). The fungal culture revealed a higher prevalence of Aspergillus niger (45.0% and 20.6% in high and low exposed regions respectively, p=0.008). Table 5 showed that the prevalence of fungi was lower than that of bacteria, and the fungal species were predominantly Aspergillus niger, Penicillium and mixed species, while the bacterial species were predominantly Klebsiella and No growth.
and 7.9% of low exposed workers showed significant growth and they were gram –ve bacteria (Klebsiella pneumoniae) (p=0.04). Fungal culture was demonstrated in 85% and 60.2% of sputum culture of high and low exposed workers. Among the high exposed workers; it was found that 45% of them were Aspergillus niger, 25% were Aspergillus flavus, 10% were penicilium and mixed growth was found in 5%. Among the low exposed workers; mixed growth was found in 29.6% while 20.6% showed Aspergillus niger and 7.9% showed Aspergillus flavus.

**DISCUSSION**

Bioaerosols have been found in many occupational environments, including animal feeding houses, poultry slaughter houses, and cotton textile plants (Su et al., 2002). In early studies, research to control byssinosis focused on methods to reduce the trash in textile mill environment. Dust control has been effective in reducing the prevalence of such condition, but simple reduction in dust levels does not always assure its prevention. Also, bacteria and fungi present in cotton do not in themselves cause byssinosis, but the protein complexes contained in their cell walls are responsible for the development of this respiratory disease of workers on cotton, flax, and some other fibers (Hend et al., 2005).

The present research studied the concentration of bacteria and fungi in cotton dust and the effect of long term exposure to high concentration of cotton dust on lung function and developing of respiratory symptoms among exposed workers in an Egyptian textile factory.

The current study revealed that cotton dust is more strongly associated with chronic airflow limitation showed in the increased incidence of respiratory symptoms including chronic cough, chronic sputum, progressive dyspnea and chest wheeze among exposed workers which came in agreement with the results reported by Noweri et al. (1990) and Madsen et al. (2012). Moreover, the incidence of symptoms among carding workers who were more exposed to high concentration of cotton dust was higher, and this is paralleled the finding of Fishwick et al. (1994), Baron et al. (2013) and Altin et al. (2002). Abnormal chest x-ray findings mainly the increase in bronchovascular marking and hyperinflation patterns were observed in a high proportion of exposed workers (30% of high and 31.8% of low exposed groups). There are some evidences that cotton workers have mucus glands hypertrophy, increased bronchial smooth musculature but nothing to suggest that they suffer from excess emphysema, either pathologically or physiologically (Pratt et al., 1980; Honeybourne et al., 1996). In an experimental study on an animal model, Milton et al. (1990) demonstrated that cotton dust includes respirable particles containing endotoxins and lactase agent, a proteolytic enzyme that is associated with the pathogenesis of emphysema.

The analysis of global alteration of pulmonary function results of the studied groups suggests an obstructive pattern among exposed workers. A decline in lung function has been more associated with high exposure than low exposure workers; the former had significantly lower FEV1, FVC/FVC% and PEFR compared to the latter values. Several studies have shown a progressive decline in ventilatory pulmonary function in exposed workers (Madsen et al., 2012; Altin et al., 2002; David et al., 2001; Hayes et al., 1994).

The present study revealed a significant decline in the mean values of FEF 25–75% indicating a small air obstruction in exposed workers and a more decrease was noticeable among high exposure workers. Small air way obstruction among cotton dust workers was also observed by Venkatakrishna et al (2001) who explained this by the elevated level of histamine among exposed workers. Moreover, Hayes et al (1994) reported that cotton workers had significantly greater decline in FEV1 (but not in FEF 25–75%) than silk workers indicating that spirometric measures for small airway function (e.g. FEF 25–75%) add little to FEV1 and FVC making air flow limitation more obvious.

Many Factors contribute to chronic respiratory symptoms and changes in lung functions among cotton dust workers. The most important factor seems to be the raw fibers, as this material always being contaminated by fungi and Gram negative bacteria. Microbiologic air sampling was performed denoting that the mean concentration of total fungi (1215 cfu/m$^3$) and isolated Aspergillus niger (993 cfu/m$^3$) measured at carding regions (highly dusty area) exceeded the WHO guideline value of 500 cfu/m$^3$ (WHO, 1990) and the concentration of other organisms was lower than this limit. A striking rise in the concentration of Aspergillus niger was recorded in carding than in spinning areas (993 and 243.8 cfu/m$^3$ respectively). Similarly, Sichletidis et al. (2004) demonstrated a higher concentration of Aspergillus niger valued 176 cfu/m$^3$ in carding region compared to 140 cfu/m$^3$ in spinning region.

Inhalation of cotton dust has been shown to lead to a neutrophilic response in air ways, probably due to a lipid fraction of bacterial cell walls and fungal active particles. Release of leukotriens and platelet activating factor by these cells plays a role in the pathogenesis of this disease condition (byssinosis) (Noman et al., 2013). In the present study, the majority of positive sputum cultures showed fungal growth which was more prevalent among high exposed workers.

Limitations of this study included the small number of the high exposed workers (n=40) in addition to the small number of significant bacterial growth culture (n=16) from the sputa of the workers. Therefore further researches are necessary to validate the results of this study.
CONCLUSION AND RECOMMENDATIONS

There is still unprotected and continuous exposure to cotton dusts, endotoxins, chemicals due to chemical and mechanical processes and to ergonomic hazards in the cotton industry in Egypt, resulting in different types of public health problems among long term exposure cotton workers. Chronic respiratory illnesses including chronic byssinosis and impaired lung function were observed among highly exposed workers of early processes of spinning (carders and blowers) as the early stages of textile processing seem to generate high fungal and bacterial contamination. Priorities should be given to occupational hygiene programs for workers at various sites in textile factories. Further work is needed to clarify potential reversibility after cessation of exposure, and the relative contributions of dust, endotoxin, and tobacco to chronic respiratory impairment in cotton dust exposed workers.

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