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Assessment of inflammatory status and oxidative stress in readymade garment manufacturing workers of Garden Reach-Metiabruz area of Kolkata

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ABSTRACT

Objectives: The textile industry including readymade garment manufacturing contributes substantially to the foreign exchange earned by India. More than 45 million people are employed in this industry. Our previous study revealed a compromised lung function (obstructive and mixed) in readymade garment manufacturing workers in the Garden Reach-Metiabruz region of Kolkata. The present study was undertaken to evaluate the impact of the work environment on the immune health at the local and systemic levels of the workers of this region. Oxidative stress encountered by the workers was also assessed.

Materials and Methods: Male workers of age group 18–35 years ($n = 80$) from readymade garment manufacturing units of Garden Reach-Metiabruz region of Kolkata, India were selected for the study. The control group was selected from the same region unexposed to the factory environment. Blood samples were collected from both groups for estimation of cytokines, C-reactive protein (CRP), cortisol and anti-oxidants of the subjects. Sputum samples from dust-exposed workers were studied for alveolar macrophages.

Results: Accumulation of alveolar macrophages in the sputum was noted in the workers which indicated a local inflammation. A systemic inflammatory state was revealed by elevated proinflammatory cytokines and CRP. Reduction in antioxidants noted is an indicator of oxidative stress in the workers.

Conclusion: A chronic proinflammatory condition exists in these workers and may be the underlying cause of the compromised respiratory status noted in the workers of this region. The inflammatory condition may lead to the pathogenesis of cardiovascular disorders, autoimmune disorders, etc., later in life. This might also influence the outcome of various respiratory infections like the current COVID-19.

Keywords: Cortisol, Dust exposure, Garment manufacturing worker, Oxidative stress, Proinflammatory cytokines

INTRODUCTION

The textile industry accounted for 15% of India's earnings from export in the financial year 2019–2020 and created employment for 45 million people.[1] Workers in the readymade cotton garment manufacturing industry (a component of the textile industry) are exposed to a variety of air-borne agents, originating from organic materials and dust in the work environment. The impact of the ambient work environment on the health of the workers of this industry needs to be assessed for the sustainable growth and productivity of the industry.[2] Our previous study demonstrated a deranged pulmonary functional state with obstructive and mixed type of

functional status in the readymade garment manufacturing workers of Garden Reach-Metiabruz region of Kolkata[3] which is largely an informal sector.[4] Particulate matters (PMs) in the ambient air not only affect lung function but also is now being recognised as a causative agent for various non-communicable diseases (NCDs) such as diabetes, cardiovascular diseases, central nervous system diseases, cancer and osteoporosis.[5,6] Chronic inflammation due to the air pollutants that the workers are exposed to often results in such derangement in lung function.[7-9] The present study was done to explore the impact of the work environment on the probable mechanism of observed lung function abnormality. To do so, we looked into the immune status and oxidative stress level of garment manufacturing workers in Garden Reach-Metiabruz region of Kolkata, India.

MATERIALS AND METHODS

Determination of dust concentration in the ambient air

Ambient air quality was assessed for 7 consecutive days for dust concentration in terms of PM up to 2.5-micrometer diameter ($PM_{2.5}$) and PM up to 10-micrometer diameter (PM_{10}) by Bharat Foundation, a government-recognised air quality analysing organisation following the Central Pollution Control Board (CPCB) guidelines for Ambient Air Quality Monitoring.[10] Since, separated cotton dust, as per its classification and definition[11] cannot be assessed, we have measured the whole dust particles according to their diameter as $PM_{2.5}$ and PM_{10} following the advice of the air quality monitoring agency.

Selection of subject

Subjects of the exposed group of workers of readymade garment manufacturing units of Garden Reach-Metiabruz, Kolkata (West Bengal, India) (n = 80) were selected by simple random sampling.[12] Information about years of exposure in this job, smoking habits, frequency of smoking, family history of respiratory diseases, educational background, financial condition and use of biomass fuel for cooking were recorded by questionnaire. They were all Muslim males of Indian origin, of age group 18–35 years. About 72.5% of the workers had primary education, 8.7% had secondary and higher education and 18.75 % were illiterates. They hailed from poor socioeconomic background with 86.25% below the poverty line and 13.75% above the poverty line. None of the workers used biomass fuel at home for cooking purpose. About 33.75% of the workers used smokeless tobacco such as Khaini and Gutkha. They did not use any personal protection equipments like masks while working. Workers with known respiratory illnesses and regular smoking habits were excluded from the study.

A control group of male subjects (n = 50) was also randomly selected[12] from the residents of the same region but without any exposure to the dust particles of this garment manufacturing units. They were age-matched office workers, school or college employees, grocery shop owners, staff of small restaurants/bakeries/confectionaries, etc., of Garden Reach-Metiabruz region having no reported respiratory diseases as per a published questionnaire.[13] The study followed the principles of the Declaration of Helsinki. A letter of consent was taken from each participant declaring his voluntary willingness to act as a subject of the study and approval of the Institutional Human Ethical Committee of Harimohan Ghose College (HMGC-IHEC/AC-MRP-UGC/02/2017 dated 03.07.2017) was obtained at the outset of the study.

Venous blood was collected from the brachial vein of the subjects by a licensed blood-collecting technician with the approval of a registered Medical Practitioner following standard procedures. Blood samples were collected in two separate test tubes: One with anticoagulant and another without anticoagulant. Serum and plasma were separated for the assays of cytokines, C-reactive protein (CRP) and oxidative stress parameters. Saliva was collected for Cortisol assay. All samples for the study were collected between 8 and 9 am.

This observational cross-sectional study was conducted during the winter months of November to January of 2017–18.

Staining and counting of alveolar macrophages

Sputum samples were collected in a container after vigorous coughing from the dust-exposed subjects. A wet cough mixed with saliva was obtained from the control group of subjects after vigorous coughing. The sputum samples from the exposed group and wet cough mixed with saliva from the control group were smeared on clean glass slides and stained by Papanicolaou stain following the method of Lahiri *et al*.[14] Briefly, smears were fixed in ether-alcohol (1:1) for 30 min before staining with Harris' hematoxylin for 2 min, followed by orange-G for 2 min and EA-50 mixture for 2 min with intermittent washing in 95% alcohol.

Stained slides were coded and scored blindly 3 times. The average of the mean values was then calculated. Alveolar macrophages, bronchial epithelial cells and sputum neutrophils, eosinophils and lymphocytes were identified by standard criteria.[15]

Determination of stress

(a) Salivary cortisol assay was done by enzyme-linked immunosorbent assay (ELISA) kit purchased from SALIMETRICS (CA, USA) as per the manufacturer's protocol. (b) The activity of the enzyme superoxide dismutase (SOD) (E.C.1.15.1.1) and Catalase (E.C.1.11.1.6) in serum

and plasma content of reduced Glutathione (GSH) were assayed by spectrophotometric methods.[16-18]

Determination of cytokines and CRP level

Serum interleukin (IL)-1β and IL-6 and whole blood interferon-gamma (IFN-γ) were assayed by ELISA kit from Raybiotech, USA. Serum CRP was assayed by an ELISA kit from Invitrogen BioSciences (India). ELISA was done with the help of a fully automated ELISA Reader (Bio-Rad, USA) as per the manufacturer's protocol. All samples were assayed in duplicate.

Statistical analysis

Standard statistical protocols were followed in the study, using MINITAB/SPSS Software. Student's *t*-test of significance was performed and the level of significance for the difference between sample means was taken as $^*P < 0.05$.[19]

RESULTS

Dust concentration in the ambient air inside the garment manufacturing unit is significantly higher than in the adjoining area

The overall dust concentrations in terms of PMs PM_{10} and $PM_{2.5}$ were measured consecutively for 7 days inside the garment manufacturing unit in collaboration with Bharat Foundation, an air-quality monitoring organisation authorised by the Government of India.

The mean values of PMs were significantly higher [Table 1] inside garment manufacturing units in comparison to the mean levels of the PMs in the adjacent area outside the garment manufacturing unit. However, the PM concentration in the garment manufacturing unit, as well as the adjoining locality, exceeded the permissible limit of 100 μg/m^3 (PM_{10}) and 60 μg/m^3($PM_{2.5}$) as prescribed by the CPCB, India.[10]

Table 1: Comparative air quality at work environment of garment manufacturing unit and the adjoining regions of the manufacturing unit.

Size of PM	Region	Concentration (μg/m^3)
PM_{10}	Adjoining areas of Garment manufacturing unit	188.3±8.2
	Garment manufacturing unit	593.4±11.5*
$PM_{2.5}$	Adjoining areas of Garment manufacturing unit	82.6±4.8
	Garment manufacturing unit	135.4±4.6*

PM: Particulate matter. Values are mean±standard error of the mean for 7 days' values. "*t*"-test was done and $^*P<0.05$ was considered to be significant

High alveolar macrophage count in sputum of dust-exposed garment manufacturing workers

Abundant alveolar macrophages (26.3 ± 1.3**) were observed in the sputum of the dust-exposed garment manufacturing workers ($P < 0.001$). In the control groups on the other hand, alveolar macrophages (2.5 ± 0.3) were very few in the wet cough mixed saliva produced by forceful coughing as shown in the photomicrographs [Figure 1].

Upregulated proinflammatory cytokines in the blood of dust-exposed garment manufacturing unit workers

The levels of proinflammatory cytokines assessed in this study namely, IL-1β, IL-6 and IFN-γ [Figure 2] were significantly increased in the exposed group in comparison to the control group reflecting a chronic systemic inflammation in the garment manufacturing workers.

Elevated CRP and salivary cortisol in exposed garment manufacturing workers

Estimation of CRP level in plasma showed a significant increase in the dust-exposed group compared with control [Figure 3]. Cortisol in the saliva was also upregulated in the dust-exposed garment manufacturing workers in comparison to unexposed controls [Figure 3].

Inhibition of antioxidant status in dust-exposed garment manufacturing workers

Activities of two enzymes involved in the disposal of reactive oxygen species (ROS) namely, SOD and catalase were measured in the serum. At the same time, content of another important antioxidant agent present in the blood, reduced GSH was also measured from the plasma of the subjects. It was found that activities of both of the antioxidant enzymes namely, SOD and Catalase were significantly decreased in the dust-exposed garment manufacturing workers in comparison to control subjects [Figure 4].

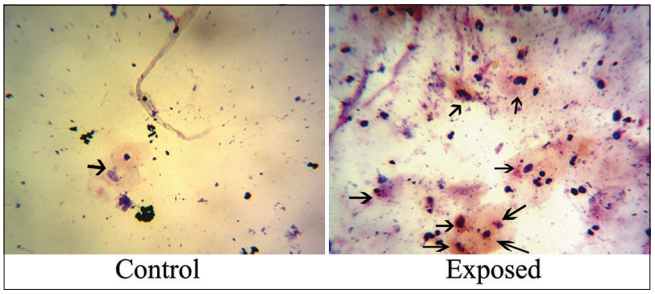

Figure 1: Photomicrographs of Papanicolaou stained smears of sputum of dust-exposed workers (right panel) and wet cough mixed with saliva from control subjects (left panel). Arrows indicate macrophages (Magnification: ×400).

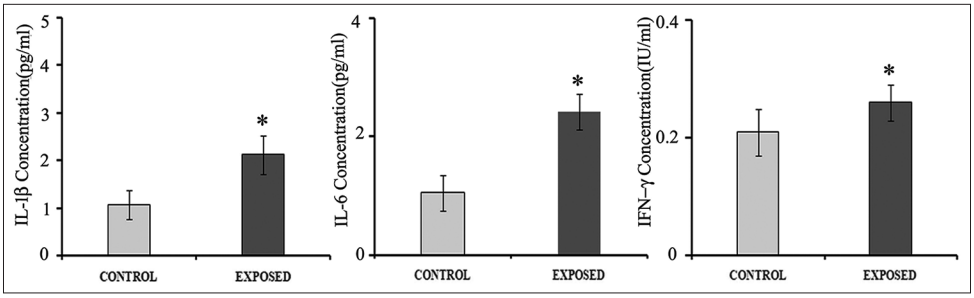

Figure 2: Proinflammatory cytokine (Interleukin [IL]-1β and IL-6 and interferon-gamma [IFN-γ]) levels in the systemic circulation of garment manufacturing workers compared to unexposed controls. Values are mean ± standard error of the mean (*$P < 0.05$).

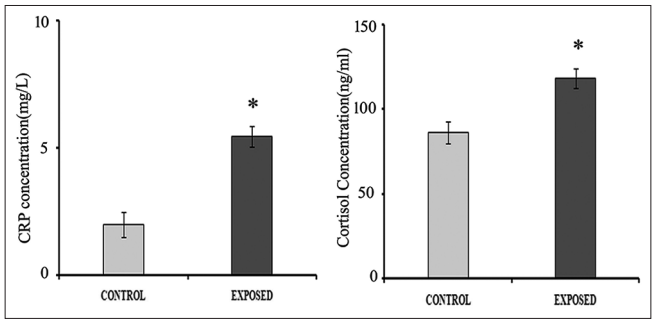

Figure 3: Chronic stress markers, serum C-reactive protein (CRP) and salivary cortisol levels in garment manufacturing workers compared to unexposed controls. Values are mean ± standard error of the mean (*$P < 0.05$).

The plasma-reduced GSH content of the exposed group was also significantly reduced compared to the control [Figure 4].

DISCUSSION

In the present study, we noticed a significant number of macrophages in the sputum of workers in garment manufacturing units. There were very few macrophages in the saliva mixed with wet cough produced by unexposed controls. The study of sputum cytology is a reliable, cost-effective and non-invasive procedure for the assessment of respiratory health. Sputum is representative of the upper as well as the lower part of the airway and contains alveolar macrophages along with neutrophils, lymphocytes and monocytes under inflammatory conditions. Sputum can be regarded as the manifestation of the inflammatory response of the lung to air pollutants. An accumulation of macrophages in the sputum of the workers suggests an inflammatory condition existing in the lungs.[20] Lung macrophages play a crucial role in the processing and removal of any inhaled PM by producing proinflammatory mediators which produce local and systemic inflammatory responses.[8]

We found significantly elevated levels of proinflammatory cytokines such as IL-6, IL-1β and IFN-γ in the cotton dust-exposed workers. IL-6 and IL-1β are often increased following occupational exposures to PMs.[21,22] IL-6 is a proinflammatory cytokine that is normally expressed at low levels, surging during infection, trauma or other stress. This cytokine has been found to be stimulating proliferation in primary human lung fibroblasts[23] suggesting that heightened IL-6 in the exposed workers might lead to fibrosis in the lung. Fibrosis might be the reason for compromised lung function (reduced forced expiratory volume in the first second) in garment manufacturing workers in this area as reported previously.[3] The elevated levels of serum IFN-γ and IL-1β might lead to poor pulmonary functional status.[24,25]

These systemic inflammatory cytokines can lead to the production of acute phase proteins and that could be important in the pathogenesis of the pulmonary and cardiovascular diseases associated with PMs.[26] Estimation of CRP showed a significant increase in dust-exposed garment manufacturing workers compared to controls. We also noted an increase in the salivary cortisol which is a classical marker of stressful conditions.[27] A study that reported that sustained stress triggered the up-regulation of glucocorticoids with an increase in proinflammatory cytokine levels contributing to various diseases[28] corroborates our observation. It appears that IL-6 may be instrumental in the upregulation of both of these markers.[29,30]

Our data also show a decreased SOD and catalase activity and a reduced GSH level, all of which are indicators of a deficient and weakened antioxidant system in the workers of garment manufacturing units leading to oxidative stress. The decrease in anti-oxidants can be by the inactivation of enzymes involved in the disposal of the ROS such as SOD and catalase, and/or by conditions that cause low levels of anti-oxidants, such as GSH[31] by PMs. Our data corroborate with the findings of Suryakar *et al*. who reported a decreased activity of SOD and catalase[32] in the workers of textile industries.

In our study, we found that the PMs, PM_{10} and $PM_{2.5}$ in the work environment far exceeded the permissible limits of 100 μg/m^3 (PM_{10}) and 60 μg/m^3 ($PM_{2.5}$).[10] Airborne PM from diverse sources has been reported to cause local (in the lungs) as well as systemic inflammation, explaining the association of

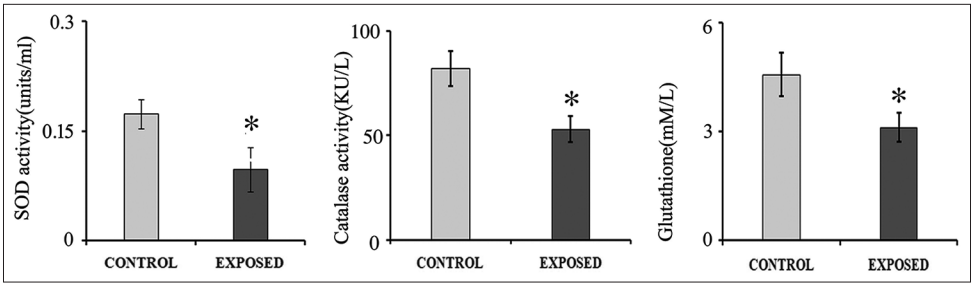

Figure 4: Activities of superoxide dismutase (SOD), catalase and content of reduced glutathione in garment manufacturing workers compared to unexposed controls. Values are mean ± standard error of the mean (*$P < 0.05$).

cardiopulmonary diseases and other auto-immune disorders like rheumatoid arthritis with these exposures.[6,33,34]

CONCLUSION

The current cross-sectional study reveals that the work environment has an impact on producing a chronic inflammatory state and oxidative stress in garment manufacturing workers. This might lead to the genesis of NCDs such as hypertension, diabetes and auto-immune disorders since inflammation is one of the leading factors in the pathogenesis of NCDs.[6,35] In addition to the development of NCDs, the fact that garment manufacturing workers have compromised respiration, a chronic proinflammatory status can have serious complications following bacterial or viral infections of the respiratory tract like the current COVID-19. An improvement in the working conditions and the use of personal protective measures like masks can be beneficial in preventing the chronic inflammatory status of the workers.[2] To achieve its goal of sustainable production, the industry immediately needs to assess the health risk to its workers and take necessary steps to control it.

However, the present study has a few limitations. The superoxide anion and hydrogen peroxide levels in the blood could have been assessed along with the antioxidant levels. Furthermore, the impact of seasonal variation on the ambient air quality[36] and biochemical markers studied here was not assessed due to financial constraints. Further, longitudinal studies are required for assessing the long-term impact of the elevated proinflammatory cytokine milieu on the health of the workers.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

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