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Original Article

Immune Functions of the Garment Workers

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Abstract

Background: Occupational exposure to cotton dust, fibers, metal fumes and different chemicals used in the apparel manufacturing industries cause a wide range of physical and psychological health problems in the garment workers that may also affect their immune function.

Objective: To assess the immune system function in garment workers.

Methods: A total of 45 workers of a garment factory, and 41 control subjects, not exposed to the garment working environment were enrolled in this study. In the study subjects, the complement system function was assessed as bactericidal activity on *Escherichia coli* DH5α cells using the standard plate count method. Serum complement components C_3 and C_4 were measured by immunoprecipitation, and IgG was measured by immunonephelometry.

Results: The bactericidal activity of serum complement in the garment workers (range: 93.5%–99.9%) was significantly ($p<0.01$) lower than that in the controls (range: 98.6%–100%). The heat-inactivated serum of the workers showed a significantly enhanced bactericidal activity. In the garment workers, the mean levels of complement C_3, and C_4 were 1.75 and 0.26 g/L, respectively that were close to those of the controls. The mean IgG level in the garment workers was 13.5 g/L that was significantly ($p<0.001$) higher than that in the controls.

Conclusion: Working in a garment factory may affect the immune system.

Keywords: Clothing; Immune system; Complement system protein; Immunoglobulins

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Introduction

The garment manufacturing process is a labor intensive task and a promising step towards industrialization of a country. At present, there are about 4500 garment factories in Bangladesh in which 4.5 million people are working. Women are main workers in this industry and make almost 80% of the total workers. With the expansion of ready-made garment industry in the 1980's, more jobs were created for poor Bangladeshi women. The sector is estimated to have provided additional direct and indirect employment to 10 million workers in related industries, such as textiles and consumer goods and services.[1] Although 76% of the export earning of Bangladesh comes from the garment manufacturing sector, the workers of these industries suffer from work-related stress and depression.

The mental workload associated with garment work is determined mainly by the complexity of the task and its speed. A high level of mental activity, visual attention and precise movements, in which eyes, hands and feet must be constantly

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TAKE-HOME MESSAGE

- Although the serum C_3 and C_4 levels of garment workers do not significantly differ from the control group, the function of the complement system in garment workers is significantly lower than the control group.
- IgG levels of garment workers are significantly higher than the control group. It seems that occupational exposure to fiber particles which are not pathogenic may stimulate antibody production.

coordinated, is required. An increased mental workload may represent a source of psychological stress. Under stress, complex adaptive mechanisms are activated and several parts of the endocrine system react simultaneously. Prolonged activation of the adaptive mechanisms is believed to be involved in the genesis of various chronic disorders such as cardiovascular, gastrointestinal, and musculoskeletal diseases.[2] The immune response is also regulated by the neuroendocrine system and events occurring in the central nervous system also modulate immune functions. Worldwide, researchers have not given due attention to garment and apparel workers. We, therefore, conducted this study to assess the immune functions of garment workers in Bangladesh.

Materials and Methods

Study subjects

A total of 45 workers of a garment factory who worked for 10–14 hours per day at Mirpur, located in northern part of Dhaka city, was enrolled in this study. There were 14 men and 31 women among the workers. Another group of 41 healthy subjects—21 men and 20 women—not exposed to the garment working environment, were enrolled as the control group.

Data collection on questionnaire

After taking informed consent, the researchers with the help of a public health nurse interviewed all the study participants. The information on work-related health problems (if any), duration of work, anthropometric parameters including age, height, and body weight of the subjects were recorded on preformed questionnaires.

Blood sample collection

From each participant, a technician from Bangladesh Medical College Hospital collected 3 mL blood samples in fresh, sterile universals. The blood sample was allowed to clot and serum was separated immediately after collection. The sera were then stored at -80 °C until analyzed.

Assay of complement mediated bactericidal activity

Escherichia coli DH5α were grown in nutrient broth for 14 hour at 37 °C in an orbital shaker. The bacterial cells were harvested, washed two times using excess of PBS and then the suspension was adjusted to 0.600 OD at 620 nm. Immediately, aliquots of 200 µL of the bacterial cell suspensions (BCS) were taken into separate tubes; 20 µL of serum was added to each tube and the mixture was incubated for 30 min at 37 °C. At the end of incubation, the remaining viable cells were serially diluted with PBS to 1:10 000. An aliquot of 20 µL of this dilution was spread on each of three agar plates and incubated for 16 hours at 37 °C. The number of colonies formed was counted and the mean value for each serum was taken from the readings of the three plates. For the negative control experiments, 20 µL of PBS

(medium) was added to the BCS instead of serum, incubated and then serially diluted with PBS to 1:50 000. An aliquot of 20 µL of this dilution was spread on each of three agar plates.

Assay of complement inactivated bactericidal activity

Complement proteins were inactivated by heat treatment at 56 °C for 30 min in a water bath and then used (20 µL) to test for bactericidal activity. Both the PBS (control) and bacteria treated with inactivated serum preparations were serially diluted to 1:50 000. The rest of the procedure was as described before.

Calculation of bactericidal activity

Bactericidal activity was calculated using the following formula, as described elsewhere.[3-5] For the control, if the mean colony-forming unit (CFU) on the plate was N_C, then 1 mL of the original bacterial cell suspension contained $N_C \times 50 \times 50\,000$ CFU.

For the test serum, if the mean CFU on the plate was N_S, then 1 mL of the bacterial cell suspension treated with serum complement contained $N_S \times 50 \times 10\,000$ CFU. Therefore,

$$\%\ \text{bacterial activity} = \frac{(N_C \times 50 \times 50\,000) - (N_S \times 50 \times 10\,000)}{N_C \times 50 \times 50\,000} \times 100$$

The bactericidal activity (%) of the inactivated serum for a mean CFU of N_i, was then

$$\frac{(N_C \times 50 \times 50\,000) - (N_i \times 50 \times 50\,000)}{N_C \times 50 \times 50\,000} \times 100$$

Determination of serum complement C_3, and C_4 and IgG

Quantitative estimates of serum complement components, C_3 and C_4, were performed using a TURBOX plus analyzer (Orion Diagnostica, Finland). The method was based on the principle of immunoprecipitation reaction of a specific antibody with its antigen. The light scattering caused by antigen-antibody complexes was measured after incubation. The intensity of the scattered light was directly proportional to the concentration of the tested complement protein present in the serum sample.

To find out if IgG is responsible for enhanced killing of bacteria after complement inactivation, IgG levels were measured in fresh sera of participants. IgG was measured by immunonephelometry using DADE Behring reagents (USA) and an autoanalyzer. The procedure was according to the supplier's recommended protocol. The results were expressed in g/L.

Statistical analysis

Data were analyzed by SPSS® for Windows® ver 17.0. Independent-samples *Student's t* test was used for comparison of means of two groups (garment workers and control subjects); Pearson correlation coefficient was used to assess extent of linear correlation between two continuous variables. A $p<0.05$ was considered statistically significant.

Results

Baseline characteristics of the study subjects

Almost 70% of the garment workers were females. The age of the garment workers ranged from 12 to 28 (men: 14–28; women: 12–25) years; the mean±SD body mass index (BMI) was 19.0±2.4 (men: 18.9±1.9; women: 19.1±2.7) kg/m². The age of the control group ranged from 20 to 35 years; they had a mean±SD BMI of 21.3±1.6 kg/m². Among the garment workers, 9% had no education, 14% could only write their names, 42% studied up to grade 3, and the remaining had

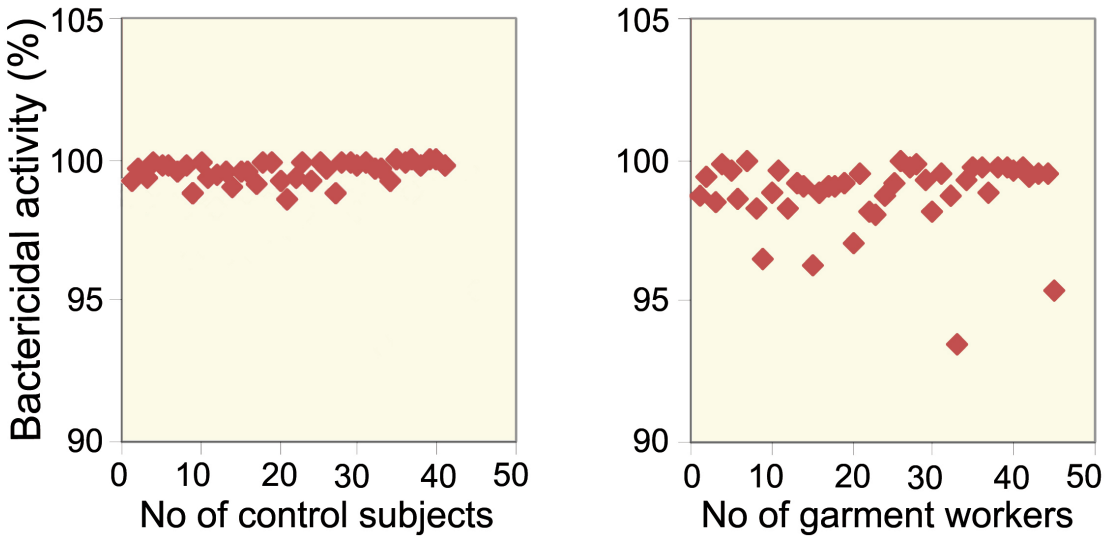

Figure 1: Percentage bactericidal activity on *E. coli* DH5α cells by serum complement from the control subjects and garment workers. Each point represents the bactericidal activity of one individual expressed as the mean value from three separate plates. The garment workers had a significantly ($p<0.01$) lower bactericidal activity compared to the control subjects.

higher education (either primary school or higher classes). The monthly income of the garment workers varied from Taka 1800–8400 with a mean value of Taka 3200 (about US$ 50). Their mean service duration at the garment factory was 2.2 years (range: 3 m to 10 yrs).

Complement mediated bactericidal activity

The number of *E. coli* DH5α colonies grown on agar plates without treating the bacterial cell suspensions with serum complement (PBS alone, negative control), varied from 307×10^6 to 800×10^6 CFU/mL. On the other hand, the number of colonies formed after treating the BCS with serum complements from control healthy subjects varied from 0.16×10^6 to 10×10^6 CFU/mL; after treating with serum complements from garment workers the number of colonies varied from 0.5×10^6 to 34.7×10^6 CFU/mL. Compared to the negative control experiments, complements from both healthy subjects and garment workers exhibited significant ($p<0.01$) bacteriolytic effects. However, the complement mediated bactericidal activity of the garment workers was significantly ($p<0.01$) lower than that of the

Table 1: Complement-mediated and complement inactivated bactericidal activities in the serum of garment workers and control subjects

% Bactericidal activity (Mean±SD)	Garment workers n=45	Control subjects n=41	p value
Complement mediated	98.8±1.3 range: 93.5–99.9	99.5±0.4 range: 98.6–100	< 0.01
Complement inactivated	33.5±9.9 range: 15.7–51.3	26.1±5.3 range: 12.8–33.1	< 0.001

Table 2: Serum levels of complements, C_3 and C_4, and IgG in the garment workers and control subjects

Concentration in serum: (Mean±SD) g/L	Garment workers n=45	Control subjects n=41	p value
Complement C_3 NV= 0.9-2.1	1.75±0.58 range: 0.51–2.71	1.73±0.50 range: 0.31–2.79	NS
Complement C_4 NV= 0.1-0.4	0.26±0.12 range: 0.09–0.80	0.23±0.08 range: 0.09–0.40	NS
Immunoglobulin G NV= 7.0-16.0	13.5±1.7 range: 10.4–18.1	11.4±2.0 range: 8.2–16.4	<0.001

NV= normal value, NS= not significant

control subjects (Table 1, Fig 1).

Effect of complement inactivation on bactericidal activity

The bactericidal activity of the complement-inactivated serum taken from the garment workers was significantly ($p<0.001$) higher than that taken from the control group (Table 1).

Levels of complement components, C_3, and C_4 and IgG

About 67% of garment workers had a normal level of serum C_3 (normal range: 0.9–2.1 g/L); 29% of the workers had above normal and only 4% had below normal C_3 levels. On the other hand, almost 78% of the control subjects had normal C_3 levels; 20% had above normal, and the remaining 2% had below normal C_3 levels. There was no significant difference in the serum C_3 level between the two studied groups (Table 2).

The serum C_4 level was within the normal range of 0.1–0.4 g/L in 91% of the garment workers; 7% of the workers had above normal, and 2% had below normal C_4 levels. Among the control subjects, 98% had normal C_4 level—the remaining 2% had below normal levels. There was also no significant difference in the serum C_4 level between the two studied groups (Table 2).

The mean±SD IgG level in the garment workers (13.5±1.7 g/L) was significantly ($p<0.001$) higher than that in the control subjects (11.4±2.0 g/L) (Table 2).

Discussion

Complement activation occurs via either the classical or the alternative pathway; these two pathways converge at the level of C_3 and share a sequence of terminal components. In this study, *E. coli* DH5α cells were used to test the bactericidal activity of the serum complement. Since this bacterium is a non-pathogen, no appreciable level of antibody against this bacterium should be present in the serum, and therefore, killing of the viable cells by serum would confirm that bactericidal activity should solely be complement mediated. We found that the complement mediated bactericidal activity of garment workers was significantly ($p<0.01$) lower than that of the control group. The reason for the lower bactericidal activity was not due to lower levels of complement since there were no significant differences in the serum level of the two major complement proteins, C_3 and C_4, between the studied groups.

The heat-inactivated serum was used

to test for bactericidal activity to determine if the bacterial cells were lysed by factors other than serum complements such as immunoglobulins. Heat inactivation for 30 min at 56 °C destroyed the complement without affecting the immunoglobulins. The mean bactericidal activity of the heat-inactivated serum of garment workers was significantly ($p<0.001$) higher than that of the control subjects. We also found significantly elevated levels of IgG in sera of garment workers. Garment workers constantly inhale fiber particles which although are non-pathogen can probably stimulate antibody production (including IgG); these antibodies could kill the bacterial cells when the serum complements are destroyed by heat inactivation. Stress-related suppression of the complement system mediated by various serum factors and cytokines would be another explanation for this observation. The results presented in this study clearly demonstrate altered immune functions in garment workers. Considering the small sample size used in this study, the findings should be generalized with caution. Larger studies are needed to provide better insights on the altered immune system in garment workers.

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Conflicts of Interest: None declared.

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