EFFECT OF CEMENT DUST EXPOSURE ON PHAGOCYTIC FUNCTION OF POLYMORPHONUCLEAR NEUTROPHILS IN CEMENT MILL WORKERS

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Abstract
Objectives: Exposure to cement dust can cause various occupational health problems due to its increasing incidence and long-term complications. However, the influence of cement dust on phagocytic function of polymorphonuclear neutrophils (PMNs), has not as yet been investigated. Therefore, the aim of the study was to measure the phagocytic activity of PMNs by assessing chemiluminescence (CL) response in cement mill workers and controls.

Material and Methods: In this study, 50 volunteer males, aged 25–60 years, apparently healthy and nonsmoking, were randomly selected from among cement mill workers. These workers were further classified into subgroups based on exposure duration of less than 10, 10–20, and more than 20 years. The controls were 50 healthy, nonsmoking, males who matched the study group with respect to age, height, weight, and socioeconomic status. The phagocytic function of PMNs, stimulated with opsonized zymosan, was determined by measuring CL response.

Results: The findings show a significant decrease in phagocytic activity of PMNs [PMNs OPZ p < 0.005] in cement mill workers compared to controls.

Conclusion: It is concluded that exposure to cement dust can impair the phagocytic function of PMNs which is reflected in decreased chemiluminescence response.

Key words: Phagocytic function, Chemiluminescence response, Cement dust

INTRODUCTION

The portland cement is a grey powder-like adhesive substance, consisting of calcium oxide (CaO), silicon oxide (SiO₂), aluminum trioxide (Al₂O₃), ferric oxide (Fe₂O₃), magnesium oxide (MgO) [1] as well as selenium [2], thallium [3], shale, clay, sand and other impurities [4]. Cement mill workers are exposed to dust at various manufacturing processes. Dust is generated during the quarrying and handling of raw materials, during grinding the clinker, blending, and in packaging and shipping the finished products [5]. The main routes of entry of cement dust particles into the body are the respiratory and gastrointestinal tracts [6]. The deposition of inhaled particles is influenced by the physical and chemical properties of cement as well
as various host factors. The physical properties of importance include particle size and density, shape, penetrability, surface area, electrostatic charge, hygroscopic activity and acidity or alkalinity of the inhaled agent. Cement dust particles can cause a disease due to the chemical nature of cement dust and its irritant, sensitizing and pneumoconiotic properties [7]. The high concentration and long-term exposure to cement dust constitutes a potential cause of occupational disease among cement mill workers. It has also been reported that cement dust particles could be found in various body organs including liver, spleen, bone, and blood and that they could produce different types of lesions [8]. The most frequently reported symptoms are cough, phlegm production, impairment of lung function, impaired performance of respiratory muscles [9], bronchial asthma, emphysema, chest tightness, restrictive lung disease, skin irritation, conjunctivitis, stomachache, and boils [1]. Moreover, cement dust also induces atrophic changes in the nasal and pharyngeal mucosa, tissue fibrosis, burning, itching and watery eyes, headache, fatigue [10] as well as cancer of the lung [11], stomach and colon [12–13].

A series of studies has been published which demonstrates the various health problems encountered among cement mill workers. However, few studies reported the effects of wood dust [14], asbestos fibers [15], dust storms [16], and urban dust on cellular dysfunctions, impaired proliferation, phagocytic activity and impaired defense mechanisms [17]. The point worth discussing is that the cement industry is one of the largest industries connected with global development, and millions of people have been working daily in this sector. However, the literature is lacking in reports on the basic- and cellular-level studies to highlight the effects of cement dust exposure on the functions of essential cells such as polymorphonuclear neutrophils [PMNs] which play an important role in the immune system. Therefore, the present study was designed to determine the phagocytic function of PMNs, stimulated with OPZ, by measuring chemiluminescence (CL) response in cement mill workers and comparing it with the values found for controls.

**MATERIALS AND METHODS**

**Subjects**
Over several months, the author and his research team visited cement manufacturing plants and interviewed 95 cement mill workers. A detailed clinical history was taken to determine whether they met the inclusion criteria. They were also questioned with regard to smoking cigarettes and other tobacco products. After the initial interview, 50 apparently healthy male cement mill workers (mean age 42.9±1.32 years (mean ±SEM), age range 25–60 years) were enrolled (study group) and 45 excluded from the study. These cement mill workers worked for at least 8–10 h a day for 5 days/week. The subjects were further classified into subgroups based on exposure duration: of less than 10 years, between 10 and 20, and more than 20 years. All the subjects completed a questionnaire, and anthropometric data were obtained. Prior to the study, the subjects gave their informed consent to participate.

The controls were selected in a similar manner: out of 90 persons interviewed, 50 apparently healthy males (mean age 42.1±1.43 years (mean ±SEM), age range 20–60 years) were admitted to the study. The group was composed primarily of clerical staff members, shopkeepers and salesmen. They matched the subjects with respect to age, height, weight and socioeconomic status.

**Exclusion criteria**
The subjects with gross anemia, known history of diabetes mellitus, cardiopulmonary disease, autoimmune disease or malignancy, as well as subjects with current or previous history of tobacco use (smoked or chewed), drug addicts [18], and subjects exposed in any industry that may generate dust or fumes other than cement industry, were also excluded from the study.

**Methods**

**Blood sample collection**
Approximately 8–10 ml blood was collected from each subject by venipuncture, using a disposable syringe. Blood was heparinized (10 IU/ml) to assess the phagocytic activity by measuring CL response. Each specimen bottle was labeled with the subject’s identification code number.
Zymosan opsonization

Zymosan (Sigma Chemical Co., St. Louis, MO, USA) was opsonized by suspending 50 mg in 3 ml human serum and 1 ml phosphate buffer saline (PBS). The suspension was incubated for 30 min at 37°C and then centrifuged at 300 g for 10 min. The supernatant was then removed and the pellet washed twice with 4 ml buffer. After the last washing, the pellet was resuspended in PBS at the concentration of 1.25 mg/ml and stored in the freezer until use. The concentration of opsonized zymosan was 2 mg/ml.

Luminol preparation

Luminol (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in DMSO to the concentration of 10^{-2} M and this stock solution was further diluted in PBS to 10^{-4} M prior to use.

Polymorphonuclear leukocyte (PMN) separation

PMNs were separated by using neutrophil isolation medium (NIM) (Cardinal Associates Inc., Santa Fe, USA). Heparinized blood (5–7 ml) was layered over NIM (4 ml) in a 15-ml tube and then centrifuged at 400 g at room temperature for 30 min. The leukocyte-rich plasma was carefully removed with a Pasteur pipette and transferred to a 15-ml conical centrifuge tube. The tube was filled with phosphate buffered saline (PBS) and centrifuged in a Heraeus centrifuge (Model GmbH, Osterode) at 350 g for 10 min. Then lysing buffer (E-Lyse) (2 ml) from the same company was added to lyse the residual erythrocytes, vortexed to resuspend the pellets and then centrifuged at 250 g for 10 min. The supernatant was discarded and the sediment suspended in 1 ml of 5% fetal calf serum (FCS). The cells were then counted and adjusted to the desired final concentration.

Assay

To measure luminol-enhanced chemiluminescence, Berthold AutoLumatPlus LB 953 luminometer with a constant temperature (37°C) controller (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany) was used. The device was connected to a computer. The reaction mixture consisted of 100 μl whole blood or PMN suspension and 900 μl medium containing 10^{-4} M luminol (5-amino-2,3-dihydro, 1,4-phthalazinedione) (Sigma Chemical Co., St. Louis, MO, USA) and 2 ng/ml OPZ (Sigma Chemical Co St. Louis, MO, USA). Light emission was recorded in millivolts (mV) and the readings were recorded at 1-min intervals for 30 min [18]. CL emission was quantified as the peak height in mV.

Statistical analysis

The difference between the mean values in the two groups was evaluated using Student’s paired t-test (two-tailed). It was regarded significant at p < 0.05.

RESULTS

Table 1 summarizes the results of comparing anthropometric parameters (age, height, weight) and phagocytic activity of polymorphonuclear neutrophils (PMN) between the groups of cement mill workers and controls. The two groups did not differ significantly with respect to mean age, height and weight. In the study group, the mean duration of exposure was 6.55±0.44 years (mean ±SEM, 86×782)

Table 1. Anthropometric parameters and chemiluminescence response in cement mill workers vs. controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cement mill workers (mean ±SEM) (n = 50)</th>
<th>Controls (mean ±SEM) (n = 50)</th>
<th>Difference (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>42.9±1.32</td>
<td>42.1±1.43</td>
<td>-1.90</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169.36±1.09</td>
<td>171±1.27</td>
<td>+0.95</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.22±1.62</td>
<td>81.8±2.31</td>
<td>+0.70</td>
<td>NS</td>
</tr>
<tr>
<td>PMNs Base</td>
<td>28.95±5.34</td>
<td>25.31±4.62</td>
<td>-14.38</td>
<td>NS</td>
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<tr>
<td>PMNs OPZ</td>
<td>906.17±88.74</td>
<td>1469.54±115.17</td>
<td>+38.33</td>
<td>0.005</td>
</tr>
</tbody>
</table>

NS — non-significant; values are expressed as mean ±SEM.

PMN concentration = 5×10^6 cells/ml; OPZ concentration = 1.25 mg/ml; luminol concentration = 10^{-4} M.

p < 0.005.
No significant differences in the mean values of anthropometric parameters were found between the two groups. The mean CL response in isolated PMNs was significantly lower \((p < 0.01)\) in cement mill workers than controls.

Table 2. Anthropometric parameters and chemiluminescence response in subjects with < 10 years’ exposure vs. controls

| Parameter | Cement mill workers (mean ±SEM) \((n = 10)\) | Controls (mean ±SEM) \((n = 10)\) | Difference (%) | \(p\)  
<table>
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<tr>
<td>Age (year)</td>
<td>33.0±2.53</td>
<td>33.8±0.84</td>
<td>+2.36</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.5±2.14</td>
<td>172.10±1.06</td>
<td>−0.81</td>
<td>NS</td>
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<tr>
<td>Weight (kg)</td>
<td>83.45±2.87</td>
<td>83.4±3.29</td>
<td>−0.05</td>
<td>NS</td>
</tr>
<tr>
<td>PMNs Base</td>
<td>29.83±14.84</td>
<td>45.84±16.92</td>
<td>+34.92</td>
<td>NS</td>
</tr>
<tr>
<td>PMNs OPZ</td>
<td>728.92±152.6</td>
<td>1881.78±385.2</td>
<td>+61.26</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.  
\(p < 0.01.\)

Table 3. Anthropometric parameters and chemiluminescence response in subjects with 10–20 years’ exposure vs. controls

| Parameter | Cement mill workers (mean ±SEM) \((n = 24)\) | Controls (mean ±SEM) \((n = 24)\) | Difference (%) | \(p\)  
<table>
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<tr>
<td>Age (year)</td>
<td>44.3±1.35</td>
<td>45.5±1.34</td>
<td>+2.63</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.9±1.45</td>
<td>171.0±2.07</td>
<td>+1.22</td>
<td>NS</td>
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<tr>
<td>Weight (kg)</td>
<td>81.85±2.54</td>
<td>79.5±1.67</td>
<td>−2.95</td>
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<tr>
<td>PMNs Base</td>
<td>30.0±6.45</td>
<td>22.55±5.82</td>
<td>−34.22</td>
<td>NS</td>
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<tr>
<td>PMNs OPZ</td>
<td>891.91±150.74</td>
<td>1252.7±106.94</td>
<td>+28.80</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.  
\(p < 0.05.\)

Table 4. Anthropometric parameters and chemiluminescence response in subjects with > 20 years’ exposure vs. controls

| Parameter | Cement mill workers (mean ±SEM) \((n = 16)\) | Controls (mean ±SEM) \((n = 16)\) | Difference (%) | \(p\)  
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<tr>
<td>Age (year)</td>
<td>49.6±1.96</td>
<td>47.2±2.0</td>
<td>−5.08</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.1±2.21</td>
<td>164.0±1.81</td>
<td>+3.59</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.88±3.0</td>
<td>76.1±2.59</td>
<td>−3.65</td>
<td>NS</td>
</tr>
<tr>
<td>PMNs Base</td>
<td>26.83±10.59</td>
<td>11.78±2.22</td>
<td>−127.75</td>
<td>NS</td>
</tr>
<tr>
<td>PMNs OPZ</td>
<td>1038.34±130.02</td>
<td>1105.3±141.1</td>
<td>+6.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.
capability of alveolar macrophages for phagocytosing the Staphylococcus aureus in vitro. Moreover, olivine inhalation also suppressed the alveolar macrophage phagocytosis to a lesser extent than silica.

In addition, Hermanowicz et al. [21] studied the neutrophil function and the prevalence of infections in workers occupationally exposed to organophosphate pesticides and among age- and sex-matched healthy controls. They found a marked impairment of the chemotactic function of neutrophils stimulated with zymosan-activated serum in all the groups of workers exposed to organophosphate pesticides.

Similarly, Donaldson et al. [22] conducted a study on rats exposed to clouds of the pneumoconiotic dusts including quartz, coal-mine dust, and chrysotile asbestos for 8, 32, and 75 days. For comparison, the rats were also exposed to the non-pathogenic dust of titanium dioxide (TiO₂). The bronchoalveolar leukocytes (macrophages and neutrophils) from dust-exposed and control rats were obtained by lavage and tested for their ability to migrate towards zymosan-activated serum. A marked decrease was found in the chemotactic activity of leukocytes from rats inhaling pneumoconiotic dusts, compared with controls. Moreover, TiO₂-exposed leukocytes showed some impairment of chemotaxis, but this was substantially less pronounced than that found for pneumoconiotic dusts.

Furthermore, Sliwinski et al. [23] conducted a study among workers exposed to dust and pesticides and reported that the chemotactic function of neutrophils stimulated with zymosan-activated serum was impaired in all the groups of workers. Also in our study, the phagocytic activity of PMNs stimulated with opsonised zymosan (OPZ) was decreased in cement mill workers, compared to their matched controls.

DISCUSSION

Occupational exposure to cement dust can cause various health problems. High concentration and/or prolonged inhalation of cement dust can provoke clinical symptoms and inflammatory response that may result in functional and structural abnormalities. The chemiluminescence response is the measurement of light emission during phagocytosis; the response being due to reactive oxygen species, namely the superoxide, necessary for antimicrobial activity. This response can be enhanced by the use of zymosan particles. Most studies of white cell functions have involved purification of PMNs from whole blood, which may activate the cells, thus increasing the extent of chemiluminescence, and reflects the respiratory burst of energy following particle contact at the cell surface [19].

Since literature on the effects of cement dust on the phagocytic function (CL response) of polymorphonuclear neutrophils (PMNs) is scarce, we would like to extend our discussion with available data on the cement dust and its effects on immune function. Scheuchenzuber et al. [20], in their study on mice, determined the immunologic responses to intermittent silica or olivine inhalation and reported that the animals exposed by inhalation to silica showed reduced capability of alveolar macrophages for phagocytosing the Staphylococcus aureus in vitro. Moreover, olivine inhalation also suppressed the alveolar macrophage phagocytosis to a lesser extent than silica.

In addition, Hermanowicz et al. [21] studied the neutrophil function and the prevalence of infections in workers occupationally exposed to organophosphate pesticides and among age- and sex-matched healthy controls. They found a marked impairment of the chemotactic function of neutrophils stimulated with zymosan-activated serum in all the groups of workers exposed to organophosphate pesticides.
reported that the ultra fine dust particles produced cytoskeletal toxicity in macrophages in vitro, which may lead to cellular dysfunctions such as impaired proliferation and phagocytic activity.

Also Huang et al. [16], who investigated the effects of dust storm on the phagocytosis of rat alveolar macrophages, have demonstrated that the dust storm particles are toxic to rat alveolar macrophages and impair the phagocytic function of alveolar macrophages in a dose-dependent manner, which may in turn impair the nonspecific defense function of the airway. The results of the present study revealed that exposure to cement dust, decreased the physiological activity of PMNs as measured by decreased CL response in cement mill workers. The previous studies by Scheuchenzuber et al. [20], Hermanowicz et al. [21], Donaldson et al. [22] and Slawinski et al. [23] focused directly or indirectly on the phagocytic function and dust but none of them concerned the cement mill workers.

In the present study, the phagocytic function of PMNs stimulated with OPZ was significantly decreased in cement mill workers, compared to controls (Table 1). Moreover, in the analysis by subgroups classified according to exposure duration, the CL response was also significantly decreased in cement mill workers exposed for less than 10, and for 10–20 years (Table 2–3). However, this decline did not reach the level of significance in a group of workers who were exposed to cement dust for more than 20 years (Table 4). Therefore, it is suggested that further, large-sample studies should be conducted to confirm the dose-response effects of the duration of exposure to cement dust on the phagocytic activity of polymorphonuclear neutrophils. Furthermore, the findings are of importance in that they highlight the necessity to reduce dust exposure in the cement industry. Therefore, it is advisable that mutual collaboration should be established between health officials, cement mill workers and their management to adopt technical preventive measures, such as ventilated work areas and appropriate protective equipment, in order to diminish health risk related to this exposure. It is also suggested that these workers must undergo pre-employment and periodic medical surveillance tests.

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