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Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society for Biological Sciences, Department of Entomology, Faculty of Sciences Ain Shams University.

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## Assessment of Infectious and Inflammatory changes in sputum associated with cement dust

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### ARTICLE INFO

#### Article History

Received: 24/10/2014

Accepted: 10/12/2014

#### Keywords:

Infectious

Inflammatory

Cement dust

### ABSTRACT

**Background:** sputum cytology is an important biomarker that can be used to assess infectious and inflammatory changes associated with different etiological factors. Therefore, the objective of this study was to assess the infectious and inflammatory changes associated with exposure to cement dust.

**Methodology:** In this cross-sectional cases control study, 500 apparently healthy volunteers were selected. Of the 500 study subjects, 400 were exposed to cement dust (ascertained as cases) and 100 were non-exposed (ascertained as controls). Sputum specimen was collected and subsequently demonstrated using Papanicalauo method.

**Results:** Inflammatory cells infiltrate associated with exposure to cement dust was found to be statistically significant (RR= 2.1214, 95% CI = 1.6142 to 2.7881, P < 0.0001). The risk of viral infection associated with exposure to cement dust was found to be (RR= 1.6250, 95% CI = 0.7078 to 3.7308, P = 0.2522). The risk associated with bacterial infection was (RR= 13.6350, 95% CI = 1.9091 to 97.3805, P = 0.0092). The risk associated with fungal infection was (RR= 2.2725, 95% CI = 0.2912 to 17.7315, P = 0.4335).

**Conclusion:** Exposure to cement dust is a significant risk that enhances infectious and inflammatory change in the lung of exposed individuals.

### INTRODUCTION

Environmental air pollution is a major risk factor that potentially contribute to burden of several diseases including; stroke, heart disease, lung cancer, and both chronic and acute respiratory diseases, including asthma (WHO, 2014). Outdoor air pollution is carcinogenic and associated with increased lung cancer incidence. An association also has been observed between outdoor air pollution and increase in other cancers such as, the cancer of urinary tract/bladder (WHO, 2013).

The relation between air-pollution related pulmonary health effects and several symptoms is well established. Influences of air pollution related lung effects most commonly due to the direct interaction between the external environment and internal biological systems and processes. The innate immune system is one of the first lines of defense against inhaled air contaminants and is characterized by activation of key signaling pathways and inflammatory cell recruitment to the lung (Laurel, *et al.* 2012).

The raw materials needed for the production of cement are mainly limestone and sources of silica, aluminium and iron. These are quarried, crushed and milled to a raw meal, which is heated in a kiln to approximately 1450°C to form clinker (cement base). The clinker is milled together with calcium sulphate and other additives to produce cement of different qualities (Anne Kristin, *et al.* 2011). A number of studies have found relations between aerosol exposure and adversarial respiratory health effects among cement production workers (Noor, *et al.* 2000; Ballal, *et al.* 2004; Mwaiselage, *et al.* 2004; Neghab and choobineh, 2007). Nevertheless, other studies didn't show such relations (Abrons, *et al.* 1988). Most studies have limitations because of their cross-sectional design, non-specific quantitative exposure data.

## MATERIALS AND METHODS

In this study 500 apparently healthy Yamani volunteers were investigated for the presence of inflammatory and infectious cytological evidences that might be associated with exposure to cement dust. Of the 500 study subjects, 400 were cement factory workers (ascertained as cases) and the remaining 100 were selected from outside the study area with no apparent environment air pollution. All study subjects were males, their ages ranging from 18 to 60 years with a mean age of 33 years. Cases were derived from cement industry workers, who exposed to cement dust for at least 6

hours per day, whereas, the controls were selected from individuals working and living in dust free areas. The cases were selected from workers who worked in cement factory for a period of more than one year. All participants completed a purposeful structured questionnaire on demographical factors, clinical symptoms and other potential risk factors for lung diseases.

**Sputum Sample collection:** Each study subject was given sputum container, and asked to give early morning expectorate (by deep cough) prior to food intake or tooth paste use and to receive it to the Laboratory as soon as possible. Specimens were prepared within a class 1 biological safety cabinet, the specimen was decanted in to a Petri-dish, and the purulent area was selected to prepare the smear on cleaned micro-slide. The smear was fixed immediately in 95% ethyl alcohol while it was wet.

**Sputum Specimen Processing:** The smears were stained adopting Papanicolaou staining procedure. Ethyl alcohol fixed smears were hydrated in descending concentrations of 95% alcohol through 70% alcohol to distilled water, for two minutes in each stage. Then the smears were treated with Harris' Heamatoxylin for five minutes to stain the nuclei, rinsed in distilled water and differentiated in 0.5% aqueous Hydrochloric Acid for a few seconds, to remove the excess stain. They were then immediately rinsed in distilled water, to stop the action of discoloration. Then the smears were blued in alkaline water for a few seconds and dehydrated in ascending alcoholic concentrations from 70%, through two changes of 95% alcohol for two minutes for each change. The smears were next treated with Eosin Azure 50 for four minutes. For cytoplasmic staining, they were treated with Papanicolaou Orange G6 for two minutes, rinsed in 95% alcohol and then dehydrated in absolute alcohol. The smears were then cleared in Xylene and mounted in DPX (Distrene Polystyrene Xylene) mounting medium.

**Assessment of Stained smears:** Firstly the quality of stained smears was assessed, through examination under low (10X) power using a light microscope. All included smears showed satisfactory staining quality. To avoid the assessment bias, cytological smears were labeled in such a way that the examiner was blinded to the group (case or control) of each subject. The smears were evaluated by two independent examiners.

**Data Analysis:** The data were analyzed for overall exposure to cement dust to identify effects on the epithelial cells of the lung. Relative risks (RRs) and 95% confidence intervals (95% CIs) were calculated. Data management was done using Statistical Package for Social Sciences (SPSS version 16). SPSS was used for analysis and to perform Pearson Chi-square test for statistical significance (P value). The 95% confidence level and confidence intervals were used.

**Ethical Consent:** All study subjects were consented to participation by completing the self-administered questionnaire.

**RESULTS**

Five hundred and thirty one subjects have participated in this study, of whom 400

were cases and 100 were controls. The age distribution was relatively similar among cases and controls in different age groups, as indicated in Fig 1. The bulk of the cases and controls belonged to the middle age range 21–30 years.

The overall inflammatory cells infiltrate was observed among 297/400 (74.3%) of the cases and 35/100(35%) of the controls, and the risk of exposure to cement dust in inducing of inflammatory cells infiltrate was found to be statistically significant (RR= 2.1214, 95% CI = 1.6142 to 2.7881, P < 0.0001), as seen Photomicrograph 1. Acute and chronic inflammatory cells infiltrate were observed among 252/400 (63%) and 45/400 (11.3%), respectively, compared to 31/100 (31%) and 4/100(4%) in this order, as shown in Table 1, Fig 2.

Cytological evidences of viral infection were identified among 39/400(9.8%) of the cases and 6/100 (6%) of controls, and the risk associated with exposure to cement dust was found to be (RR= 1.6250, 95% CI = 0.7078 to 3.7308, P = 0.2522), as indicated in Fig2 and seen Photomicrograph 2.

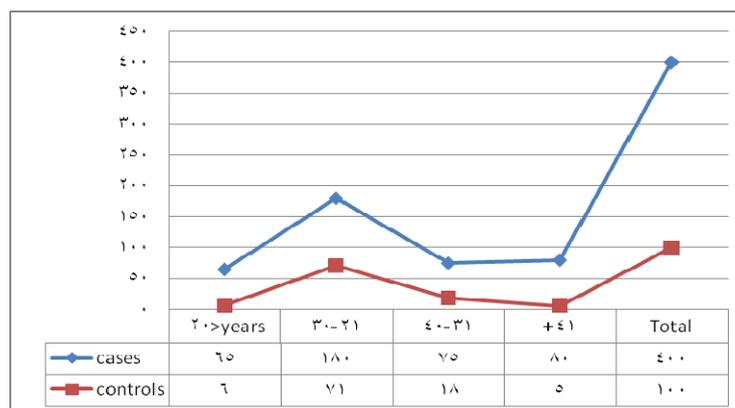


Fig. 1: Description of the study population by age.

Table 1: Distribution of the study population by Inflammatory and infectious changes.

Category	Cases	Controls	P values
Acute inflammation	252	31	0.001
Chronic inflammation	45	4	0.0425
Viral infection	39	6	0.2522
Bacterial infection	54	0	0.0092
Fungal infection	9	1	0.4335

Actinomyces (bacteria) was observed in 54/400(13.5%) of the cases and 1/100 (1%) of controls (RR= 13.6350, 95% CI = 1.9091 to 97.3805, P = 0.0092), as indicated in Fig2.

Moniliasis (fungal) was observed in 9/400(2.3%) of the cases and 1(1%) of controls (RR= 2.2725, 95% CI = 0.2912 to 17.7315, P = 0.4335), as indicated in Fig2.

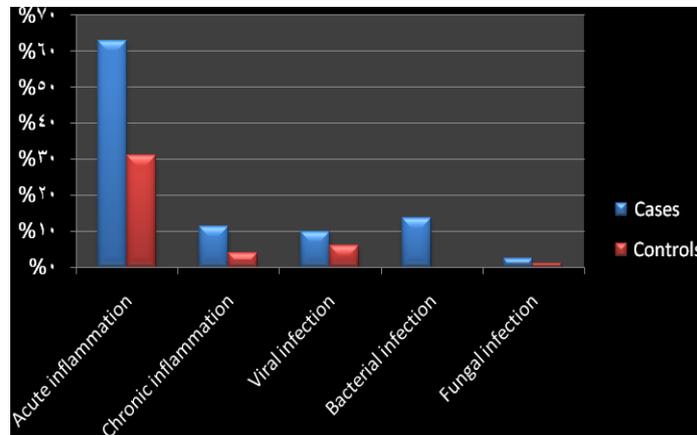


Fig. 2: Description of the study population by inflammatory and infectious conditions.

Fig. 3 describes the cases by age and inflammatory and infectious conations. However, with the exception of age group

21-30 years, proportion of these conditions were relatively similar among different age ranges, as indicated in Fig. 3.

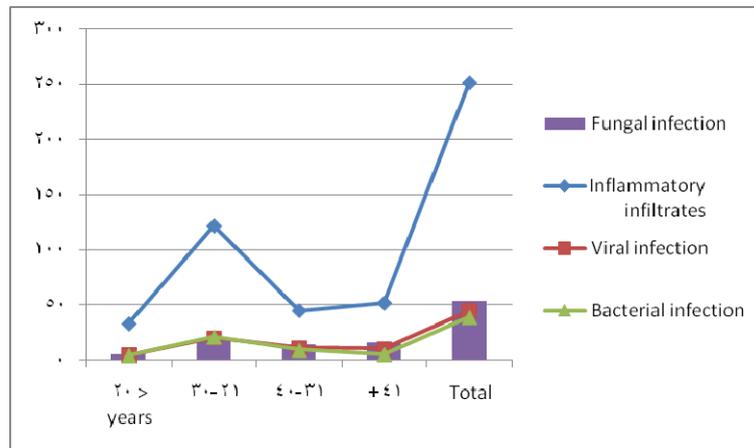


Fig. 3: Description of the cases by inflammatory and infectious conditions and age.

**DISCUSSION**

Exposure to cement is associated with increased morbidity of lung disease among exposed workers and the elevation of the risk is associated with several factors. Thus, the aim of this study was to compare lung inflammatory cells infiltrate and infectious conditions in sputum samples from cement production workers between exposed (cases) and non-exposed (controls). However, a statistically significant difference was identified in regard to the role of cement

exposure a major risk factor that promote inflammatory cells infiltrate in the lung.

However, few studies have investigated the causal physiological mechanisms involved in cement-made lung effects. Irritation of mucus membranes because of the alkaline properties of cement dust and the chance that other content particles (quartz, chromium) cause inflammation have been incriminated. In an experimental study, cement dust was found to activate macrophage tumor necrosis factor (TNF)-α

production in alveolar macrophages (Van Berlo, *et al.* 2009). Another study have observed an increase in the quantity of neutrophils and levels of interleukin (IL)-1 $\beta$  in induced sputum samples from cement production workers (Fell Ak, *et al.* 2010). In a Norwegian study, a significantly higher percentage of neutrophils was observed in cement production workers during the exposed period compared with both the non-exposed period and the external reference group, and corresponded with elevated IL-1beta concentration (Toren K, *et al.* 2010).

Furthermore, some of these inflammatory infiltrates might be also associated with infections. Acute inflammatory cells in most instances associated with bacterial infection which might be enhanced by irritation effect of cement components. In the present study a reasonable number of individuals were identified with bacteria, although some of these might be contaminants from oral cavity. Although, whether occupational exposure to agents, such as inorganic dust or chemicals, also increases the risk for infectious pneumonia is not clear, but, there are epidemiological studies indicating that exposure to metal fumes is a risk factor for infectious pneumonia (Toren K, *et al.* 2011). These studies, indicate that exposure to inorganic dust increases the mortality from infectious pneumonias, particularly lobar pneumonia and pneumococcal pneumonia. The mechanism is unclear, but the effect may be mediated through induced airways inflammation.

Cytological evidences of viral infection, as well as, susceptibility for moniliasis (fungal infection) were significantly elevated among cases compared to controls. However, how exposures to cement dust accelerate the susceptibility to these infectious agents needs further measurements.

On exposure to air pollutants and smoking, airway epithelial cells, which form the first line of innate immune defense against particles, produce inflammatory

response (Bayarm HK, *et al.* 2006). In addition, air pollutants and smoking can also cause cell death by necrosis, and by the process of apoptosis, which can increase inflammatory potency (Liv, *et al.* 2002; Alfaro-Moreno, *et al.* 2002). Some studies have reported that exposure to some air pollutants potentiates acute inflammation and mucus production and secretion elicited by a biogenic substance in rat pulmonary airways (James, *et al.* 2003). However, the same change might be induced by cement dust with enhancing of viral infection.

Although, there is possibility of contamination with *Monillia* from oral cavity, but its presence in a reasonable number in cases compared to controls, incriminates the invisible role of cement dust as a synergistic enhancing factor.

Moreover, these may clarify the presence of inflammatory cells infiltrate in significant number among cases compared to controls.

One of the causes of occupational disease is the failure of management to educate its workers on how to protect themselves from hazardous substances that are present in the workplace. Manufactures that produce cement are required to provide their workers with good quality of personal protective elements to protect them from contacting cement dust and fume while working.

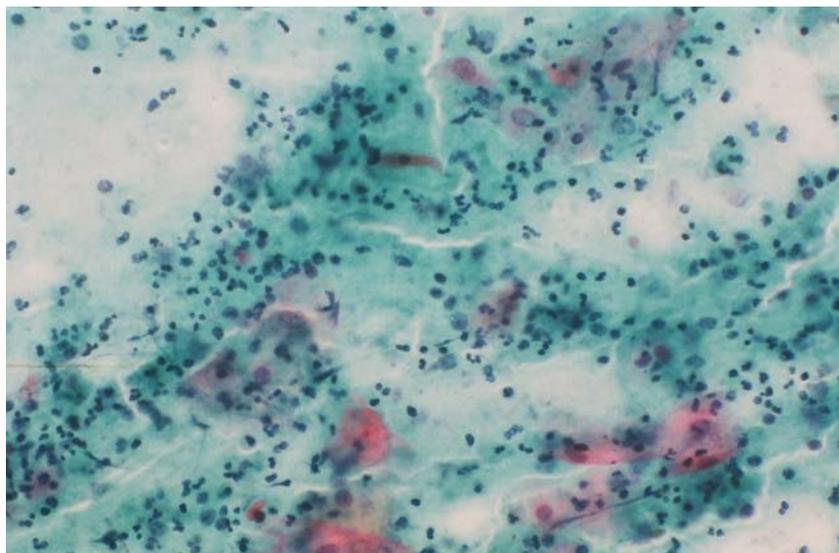
However, one of the limitation of this study, is that the number of non-exposed individuals (controls) is small (100) compared to exposed (cases) (400), which may reduce its effects. To reduce this we calculate some statistical measures for cases or controls independently.

In conclusion: Exposure to cement dust is a risk factor for lung susceptibility to infections and inflammatory changes, particularly in developing countries, where the protective measures are very low. Implementation of preventive strategies for cement dust works is important, which can include continues sputum tests, early

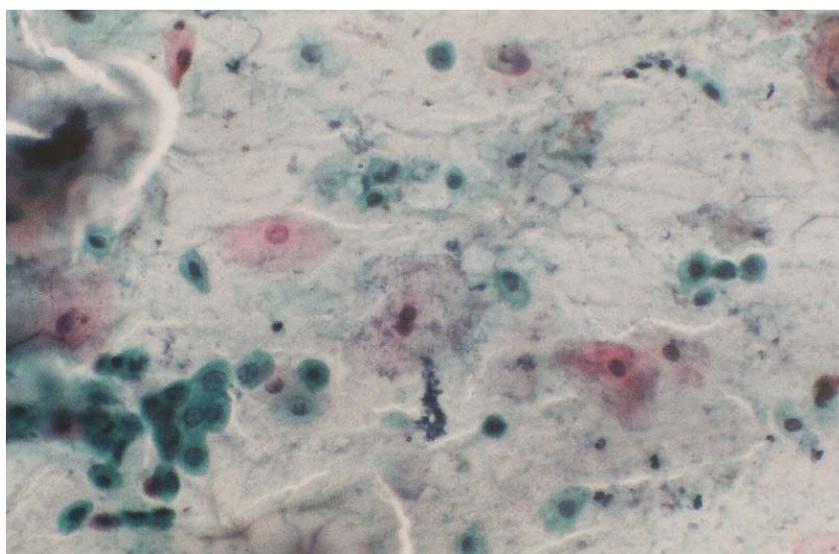
limitation of exposure, and inevitable use of protective mask during work.

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Photomicrograph 1: Sputum smear showing dense inflammatory cells infiltrate.



Photomicrograph 2: Sputum smear showing evidences of viral infection

## ARABIC SUMMARY

## تقييم التغيرات المعديّة والالتهاب اتفبال بلغم والمرتبطة بالتعرض لغبار الاسمنت

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**الخلفية العلمية:** دراسة علم الخلايا في البلغم والعلامات البيولوجية الهامة التي يمكن استخدامها لتقييم المعدي منها والتغيرات الالتهابية المرتبطة مع العوامل المختلفة والمسببه لذلك.الهدف من هذه الدراسة هو تقييم التغيرات المعديّة والالتهابات المرتبطة بالتعرض الى غبار الأسمنت.

**المنهجية:** في هذه الدراسة تم دراسة ٥٠٠ عينة من المتطوعين، منها ٤٠٠ عينة ل أشخاص تعرضوا لغبار الأسمنت (عاملين في مصنع الأسمنت) و ١٠٠ عينة من أشخاص لم يتعرضوا ل غبار الأسمنت.عينة البلغم جمعت وأظهرت لاحقاً باستخدام طريقة Pap.

**النتائج:** الخلايا الالتهابية المرتبطة بالتعرض للغبار وجدت لتكون ذات دلالة إحصائية

(RR= 2.1214, 95% CI = 1.6142 to 2.7881, P < 0.0001)

تم العثور على عدوى الفيروسات مرتبطة بالتعرض لغبار الأسمنت وكانت كالتالي

(RR= 1.6250, 95% CI =0.7078 to 3.7308, P = 0.2522)

وكانت المخاطر المرتبطة بالعدوى البكتيرية

(RR= 13.6350, 95% CI = 1.9091 to 97.3805, P = 0.0092)

كذلك المخاطر المرتبطة بالعدوى الفطرية

(RR=2.2725, 95% CI = 0.2912 to 17.7315, P = 0.4335)

**الخلاصة:** التعرض لغبار الأسمنت هو خطر كبير يعزز من التغيرات المعديّة والتهاب الرئة في الأشخاص المعرضين

له.