



## Effect of Occupational Cement Dust Pollution on The Respiratory Epithelium in Amran Cement Factory – Yemen

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### ABSTRACT

This study aimed to investigate the risk of the respiratory epithelium in regards to occupational exposure to cement dust. Detailed job histories and sputum samples were elicited from 531 individuals, who 433 individuals confirmed exposure to cement dust (cases) and 98 individuals healthy none exposed (controls). Cytological smears were prepared and demonstrated using Papnicoloua test and Silver Nucleolar organizer Region AgNORs methods. Data were analyzed using the SPSS program, mean and chi-square were calculated. The cytological changes were elevated in exposure compared to controls, the risk associated with occupational cement dust pollution was found to be statistically significant ( $p < 0.05$ ). Out of the 433 cases, the following results, 21(4.8%) atypia (dysplasia), 236(54.5%) squamous metaplasia, 252(58.2%) acute inflammatory infiltrated cells, 45(10.4%) chronic inflammatory infiltrated cells, *Actinomyces israelii* and *Monilia* were observed in 54(13%), 9(2%), respectively, cytological evidence of viral infection 39(9%). The mean AgNORs dots counts was elevated among exposure ( $3 \pm 0.043$ ) and less among controls ( $1.8 \pm 0.07$ ), was statistically significant ( $P < 0.000$ ). The study concluded that exposure to cement dust is a risk factor for occurrence of cytological atypia (dysplasia), squamous metaplasia, inflammatory changes and susceptibility of infections. The mean AgNORs counts is a useful indicator for cellular proliferation activity in respiratory epithelium and for prediction of the risk of exposure to certain carcinogenic elements that may induce lung cancer. Cytology is a useful technique in evaluation of environmental and industrial changes.

### المستخلص

هدفت هذه الدراسة للتحقق من خطر التعرض المهني لغبار الأسمنت على الظهارة التنفسية. الأدوات والأساليب المستخدمة هي عينات البلغم والتاريخ الوظيفي التفصيلي من 531 شخص، منها 433 شخص الذي أكد تعرضهم لغبار الأسمنت (المتعرضين) و 98 شخص لم يتعرضوا لغبار الأسمنت (التحكميين). وقد تم تحضير المسحات الخلوية وصيغها باستخدام صبغة البانايكولا والفضة (AgNORs). وتم تحليل البيانات بواسطة برنامج SPSS اختبار مربع كاي وكذلك المتوسط الحسابي. كانت التغيرات الخلوية مرتفعة في المتعرضين مقارنة بالمجموعة التحكمية، وكان الخطر المرتبط بتلوث غبار الإسمنت المهني ذات دلالة إحصائية ( $p < 0.05$ ). من 433 حاله المتعرضين تم التعرف على الخلايا اللانمطية (خلل التنسج) في 21(4.8%). وتحول النسيج

الحرشفي في (54.5%) 236. وكذلك الخلايا الأنتهايبية الحادة في (58.2%) 252 والمزمنة في (10.4%) 45. ولوحظ عدوى الشَّعبية الإسرائيلية في (13%) 54، والطوقية في (2%) 9. والأدلة من العدوى الفيروسية (هاله حول النواه) في (9%) 39. ووجد متوسط نقاط AgNORS مرتفع في المتعرضين (3±0.043) وأقل بين التحكميين (1.8±0.07)، وكان ذا دلالة إحصائية ( $P < 0.000$ ). خلصت هذه الدراسة الى أن التعرض لغبار الإسمنت هو أحد عوامل الخطر لحدوث الخلايا الألامطية (خلل التنسج) وتحول النسيج الحرشفي والتغيرات الانتهايبية و الحساسية للعدوى الفطرية والفيروسية وإعتد برّ متوسط عدد AgNORS هو مؤشر مفيد للنشاط الانتشاري الخلوي في الظهارة التنفسية وللتنبؤ من خطر التعرض لبعض العناصر المسببة للسرطان التي ربما تسبب سرطان الرئة. كما ان علم الخلايا هو تقنية مفيدة في تقييم التغيرات البيئية والصناعية.

**KEYWORDS:** Lung cancer, sputum cytology, atypia, air pollution

## INTRODUCTION

Lung cancer is most common in the world and is one of the five major causes of death across the global <sup>(1)</sup>. The most important risk factor is tobacco smoking <sup>(2)</sup>. Study of American Cancer Society found strong associations between air pollution and lung cancer <sup>(3)</sup>. Cement plants are important emission sources of pollution of both organic and inorganic chemicals, and produce an input of metals. There are about 17 trace elements in cement dust; antimony, arsenic, lead, cadmium, chromium, cobalt, copper, manganese, nickel, thallium, tin, vanadium, zinc, beryllium, selenium, tellurium and mercury <sup>(4)</sup>. Even in the 21 century, millions of people are working daily in a dusty environment. Cement dust causes lung function impairment, chronic obstructive lung disease, restrictive lung disease, pneumoconiosis and carcinoma of the lungs, stomach and colon diseases <sup>(5)</sup>. Silica which is a major constituent of cement dust has been mostly implicated to cause or contribute to several diseases including acute silicosis, interstitial fibrosis, rheumatoid complications, vascular disease, glomerulonephritis and immune system might be affected <sup>(6)</sup>. Most studies on long-term exposure to

air pollution and lung cancer risk have investigated the association with lung cancer mortality and lung function in world wide. However, this is the first study in Yemen, from 2011 to 2013, which aimed to find out the association between exposure to cement dust pollution and prevalence of pre-malignant, malignant and inflammatory changes using cytological method.

## MATERIALS and METHODS

### Participants

Five hundred and thirty one, sputum materials were randomly collected from cement factory workers and surrounding people in Amran City-Yemen. 433 individuals were exposed to cement dust (ascertained as cases) and 98 individuals were none exposed (ascertained as controls). Each participant was well informed about the study and he signed a written ethical consent form before participating in the study that was approved by the Ethical Committee, College of Medical Laboratory Science Research Board, Sudan University of Science and Technology. The controls were selected from individuals none exposed to cement dust and who were non smokers. The cases selected from the workers and surrounding populations were exposed to cement

dust for a period of more than one year.

### **Samples Collection**

Collection of the sputum specimen, each study subject was given sputum container, and asked to provide early morning expectorate (by deep cough) before food intake or tooth paste use and to take it to the laboratory as soon as possible. Four smears were prepared from each specimen. The specimens were prepared within a class one 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, for 15 seconds.

### **Samples Processing**

Experiment 1. Using Papanicolaou staining method described by Bancroft<sup>(7)</sup>. Quality control measures were adopted during sample collection and processing. The smear adequacy was confirmed by the presence of alveolar macrophages. Each smear was examined for atypia, squamous metaplasia, fungal infection and inflammatory cells. Atypia was assessed cytologically by using the criteria described by Ahmed *et al.*<sup>(8)</sup> The presence of two or more of the following features were consistent with Atypia: nuclear enlargement associated with increased nuclear cytoplasmic ratio, hyperchromatism, chromatin clumping with moderately prominent nucleoli, irregular nuclear membranes and bi- or multi-nucleation, scant cytoplasm, and variation in size and/or

shape of the cells and nuclei. Experiment 2. Using AgNOR staining method described by Ploton *et al.*<sup>(9)</sup> With a modification in procedure, as following, pre-staining fixation for 30 min in Carnoy's solution (acetic acid: absolute ethanol, 1:3). All smears were glass slide and then placed face down in the AgNOR obtained solution with accurate positioning, horizontal position (cover plate with a slide inverted into colloidal silver solution), and incubation for at 45°C, for 12 min in a dark place. The quantity of silver used was limited to 0.2- 0.3 ml per slide. By staining slides in a humidified chamber solution was discarded and slides were washed in several baths of distilled water. Dehydrated and mounted routinely. The AgNORs dots were counted in the nuclei of the first 50 non-overlapping. It was performed according to the technique described by Crocker *et al.*<sup>(10)</sup> The black dots inside the well-defined nuclei were counted, with aggregations (overlapping or fused black dots) being considered a single structure.

### **Statistical analysis**

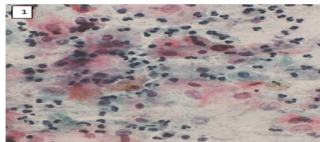
Data were analyzed using the SPSS computer program. Mean, chi-square test and independent sample test were done for statistical significance (P=value).

### **RESULTS**

Distribution of pathological findings in occupational exposure to cement dust and control group, were indicated in Table 1, and Figures (1-5).

*Table 1: Distribution of pathological findings to occupational cement dust and control group*

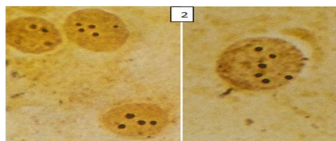
Category	Variable	Case (exposure)		Control		p-value
		NO	%	NO	%	
Cytological changes	Dysplasia	21	4.8	0	0	0.013
	Metaplasia	236	54.5	43	43.9	0.037
Inflammatory cells	Acute	252	58.2	43	43.9	0.007
	Chronic	45	10.4	4	4.1	0.032
Viral Infection	Hpv	39	9	6	6.1	0.240
Fungal infections	Actinomyces	54	13	0	0	0.000
	Monilia	9	2	1	1	0.001
Mean NOR count		3 ± .043		1.8 ± .07		0.000



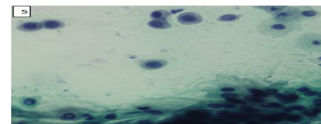
*Figure 1: Sputum smear showing dense inflammatory cells infiltrate. Papanicolaou x40*



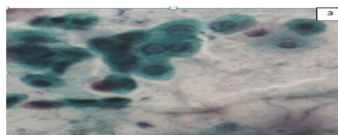
*Figure 4: Sputum smears showing Actinomyces israelii. Papanicolaou x40.*



*Figure 2: Sputum smears showing AgNOR dots. Silver stain x100.*



*Figure 5: Sputum smear showing atypia. Papanicolaou x40*



*Figure 3: Sputum smear showing squamous metaplasia. Papanicolaou x40.*

Cytological atypia (dysplasia) were detected in 21(4.8%) of the cases and was not detected in controls, the risk was found to be statistically significant ( $P < 0.013$ ). Notably, squamous metaplasia were detected in 236(54.5%) of the cases and 43(43.9%) of the controls, the risk was found to be statistically significant ( $P < 0.037$ ). Acute inflammatory cells infiltrate was observed in 252(58.2%) of the cases and 43(43.9%) of the controls ( $P < 0.007$ ). Chronic Inflammatory cells infiltrate was observed in 45(10.4%) of the cases and 4(4.1%) of the controls ( $P < 0.032$ ). Asbestos bodies were also detected in 59(13.6%) of the cases and none of the controls ( $P < 0.000$ ). The Fungal infection by *Actinomyces israelii* and *Monilia* were detected in 54(13%) and 9(2%) of the cases and one case the controls, respectively, which was statistically significant ( $p < 0.001$ ). Inanition, cytological evidence of viral infection was detected in 39(9%) which was statistically insignificant ( $p > 0.240$ ). The mean AgNORs dots counts were elevated among the cases ( $3 \pm 0.043$ ) and less among the controls ( $1.8 \pm 0.07$ ), the risk was found to be statistically significant ( $P < 0.000$ ).

#### **DISCUSSION**

This study showed stronger association of exposure to cement dust pollution with lung dysplasia and squamous metaplasia. Dysplasia is believed to be a precursor of squamous cell carcinoma. It was demonstrated in 21(4.8%) of the cases and none of controls. Squamous metaplasia is different from dysplasia and is not directly considered premalignant but it can develop into premalignant, and was demonstrated in 236(54.5%) of the cases and 43(43.9%) of the controls. Setta *et al.* <sup>(11)</sup> reported cytological abnormalities in induced sputum and increased local and systemic pre-

inflammatory status in asbestos exposure. Johansson *et al.* <sup>(12)</sup> indicates an association between the degree of exposure to asbestos and adenocarcinoma of the lung. Dietz *et al.* <sup>(13)</sup> reported an elevated risk of larynx cancer associated with occupational exposure to cement in 257 cases and 769 population controls. Yahaya *et al.* <sup>(14)</sup> reported the histopathology analysis of the lung tissues of the exposed rats as abnormal alveolar architecture, damaged bronchioles, disrupted bronchus, weak respiratory connective tissues, degenerated epithelium linings and inflammations. Huuskonen *et al.*, <sup>(15)</sup> reported 5(4.4%) with benign dysplasia, 2 with suspicious cells for carcinoma, 1 with anaplastic carcinoma and 36(31.6%) with squamous metaplasia of cytological sputum examination in 114 asbestos cement workers. Raffn *et al.* <sup>(16)</sup> reported asbestos cement work is associated with an increased risk of lung cancer of all main types. Giordano *et al.* <sup>(17)</sup> confirmed an increased risk of respiratory system cancer with previous work exposure to a cement asbestos plant. Greenberg *et al.* <sup>(18)</sup> reported of the 554, 44(8%) mild to moderate atypias, 18(3.2%) severe atypias, 2(0.4%) squamous carcinoma and 232(42%) squamous metaplasia in former asbestos cement workers. Rafnsson *et al.* <sup>(19)</sup> reported masons handling cement, a high mortality rate of lung cancer. Ahmed *et al.* <sup>(20)</sup> reported 4(3%) with atypia (dysplasia), 15(11.5%) with squamous metaplasia in cytological sputum examinations of mineral workers in Sudan. The atypical changes of lung epithelial cells can be accepted as a progression towards features of dysplastic cellular changes. It is well established that, changing of a normal cell to a malignant cell requires the occurrence of a precursor



nonmalignant cell, which exhibits increased DNA changes, cell proliferation, and apoptosis<sup>(21)</sup>. Vetrani *et al.*<sup>(22)</sup> concluded 13(3.5%) with atypical squamous metaplasia, 1(0.3%) of presumed carcinoma in situ and 54(14.6%) with squamous metaplasia in workers exposed to air pollution. Safa and Abdulh reported 2(0.4) atypical metaplastic cells and 29(5.8%) squamous metaplasia of the surrounding people exposure to cement dust in Sudan<sup>(23)</sup>. Mohammad<sup>(24)</sup> stated 35.5% squamous metaplasia in non smoker cement workers. Ahmed and Rezgalla observed dysplasia was detected in 7(2.3%) of the cases and 2(0.7%) of the controls and squamous metaplasia was detected in 58(69%) of the cases and 26(31%) of the controls in smoking and traffic-related air pollution in Sudan ( $P < 0.001$ )<sup>(25)</sup>. Oguztuzun *et al.*<sup>(26)</sup> examined heavy gun, steel and ammunition plants workers by sputum cytology they found squamous metaplasia in 33, dysplasia in 2 and 61 inflammations workers out of 199 study subjects. The mean AgNOR dots count ( $3 \pm .043$ ) of exposure has significantly higher than controls group ( $1.8 \pm .07$ ) ( $P < 0.000$ ). This indicates that, cement dust exposure increases cellular proliferative activity. No previous study used AgNOR method in sputum exfoliated cells of cement dust exposure. However, AgNORs mean counts are increased in smokers (mean=3.68) alcohol drinkers (mean=2.82), peppers and hot meals (mean=2.28)<sup>(27)</sup>. Toombak users (mean= 3.081±0.39)<sup>(28)</sup>. In this study acute and chronic inflammatory cells infiltrate were observed with a significantly increase among the exposed subject more than controls. Safa and Abdulh showed (18.9%) inflammatory changes of people cement dust exposure in Sudan<sup>(23)</sup>

while Mohammad<sup>(24)</sup> stated (53.5%) inflammatory changes in non smoker cement factory worker in Sudan. Setta *et al.*<sup>(11)</sup> reported asbestosis had reduced sputum cellularity but higher macrophage and neutrophil ratio. Fell *et al.*<sup>(29)</sup> observed an increase the numbers and percentages of neutrophils and lymphocytes in induced sputum samples from cement production workers. Also this study is consistent with the findings of previous studies which reported an association between inflammatory cells and exposure to particles derived from tobacco smoking, air pollution, and occupational exposure<sup>(27,25,20)</sup>. Cytological evidences of viral infection, as well as, for *Actinomyces israili* and *Monilia* were significantly elevated among exposure compared to controls. Ahmed *et al.*<sup>(20)</sup> found evidences of viral and Moniliasis infections in exposed groups in metallic dust iron and aluminum. The possibility of contamination with *Actinomyces israili* and *Monilia* in exposure compared to controls incriminates the invisible synergistic role of cement dust. Moreover, these may clarify the presence of inflammatory cell infiltrate among exposed workers group

## CONCLUSIONS

xposure to cement dust air pollution was associated with high elevated risks for developing lung dysplasia, squamous metaplasia, inflammatory changes and susceptibility of infections. The mean AgNORs count is a useful indicator for cellular proliferation activity in respiratory epithelium and for prediction of the risk of exposure to certain carcinogenic elements that may induce lung cancer. The present study recommended the implementation of preventive strategies for cement dust works is important. Cytological sputum tests,

early limitation of exposure, and inevitable use of protective mask during work were important which can decrease the pollution hazards exposure for cement dust plants workers.

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