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RELEVANCE OF ASBESTOS BODIES IN SPUTUM AND ASSOCIATION WITH CYTOLOGICAL CHANGES OF RESPIRATORY EPITHELIUM AMONG THOSE EXPOSED TO CEMENT DUST POLLUTION IN AMRAN CITY – YEMEN

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This is descriptive case control study, from July 2010 to July 2013, aimed to investigate of asbestos body in the sputum and the extent of their association with cytological changes in the respiratory epithelium. Sputum samples were collected from 531 individuals; of who 433 were exposed to cement dust pollution (cases) and 98 were non exposed (controls), their ages ranging from 11 to 70 years and genders participated 376/531 male and 155/531 female in the cases. Cytological smears was prepared from each sample and demonstrated by Papanicolaou method for detection of cytological changes. Data were analyzed using the SPSS program, mean and chi-square were calculated. The cytological changes were elevated in asbestos body cases compared to non asbestos body cases and controls, out of 433 cases, asbestos body was detected in 59(13.6%) of the cases and none of the controls, in addition, the cytological changes in asbestos cases were detected in 5(8.5%) dysplasia, 34(57.6%) squamous metaplasia, 41(69.5%) acute inflammatory infiltrate cells and 13(22%) chronic inflammatory infiltrate cells, Notably, high proportion of asbestos bodies were detected with long-term of duration exposed, closeness, female than male, surrounding people than workers, casing department and with alhagz residence. The study concluded the presence of asbestos bodies in sputum workers of cement is stronger factor of occurrence of cytological changes in respiratory epithelium. The asbestos fibers risks are increasing with long-term of exposed and closeness.

Keyword: Lung cancer, sputum cytology, atypia, air pollution

INTRODUCTION

Asbestos is minerals fibers that can be separated into thin, durable threads, can cause scarring and inflammation of lung, serious health problems and as a known human carcinogen (ATSDR, 2009). The asbestos is associated with cancer of the respiratory system, and several studies confirm this risk for workers of cement plant (Ulvestad *et al*., 2002; Magnani *et al.,* 2008). Exposure to asbestos may increase the risk of lung cancer, mesothelioma, asbestosis and other disease (NTP, 2009; Marbbn, 2009). The relative risk in asbestos workers to be increased up to five to six times (Gerald *et al*., 2003). 50% of male lung cancer cases that occur in industrialized countries are linked to occupation (Martin *et al*., 2000). Since the level of awareness regarding occupational hazards among factory workers and surrounding population are limited, many workers with undetected respiratory diseases may exist. Therefore, this is the first study in Yemen, from 2010 to 2013, which aimed to investigate of asbestos body in the sputum and their association with cytological changes in the respiratory epithelium using cytological method.

MATERIALS and METHODS

Participants

Five hundred thirty-one individuals share in this study, 433 were exposed to cement dust (cases) and 98 none exposed to cement dust (controls). Their ages ranging from 11 to 70 years and genders participated 376/531 male and

155/531 female in the cases. Only one sputum specimen was obtained from each subject. Cytological smears were prepared and demonstrated using Papnicoloua method. Each participant was well informed about the study and 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 cases and controls were selected from individuals which 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 smears on cleaned micro-slides. The smears were fixed immediately in 95% ethyl alcohol while it was wet, for 15 seconds.

Samples Processing

Samples Processing using Papanicolaou staining method described by Bancroft (Bancroft and Gamble, 2002). 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, inflammatory cells and asbestos body. Atypia was assessed cytologically by using the criteria described by Ahmed *et al*. (2003).

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.

Statistical analysis

Data were analyzed using the SPSS computer program. Mean, chi-square test were done for statistical significance (P=0.05).

RESULTS

In distribution of the study population case and control groups, asbestos body was detected in 59(13.6%) of the cases and none of the controls (P=0.000), as seen in Table 1and in Figures (ATSDR, 2009; Magnani *et al.,* 2008). In distribution of cytological changes according to asbestos cases, non asbestos cases and control, the cytological changes, dysplasia, metaplastic cells, acute and chronic inflammatory cellular infiltrate, which were observed in 5(8.5%), 34(57.6%), 41(69.5%) and 13(22.0%) of the asbestos cases rather than non asbestos cases which were observed in 16(4.3%), 202(54.0%), 211(56.4%) and 32(8.6%) and control which were observed in 0(0%), 45(45.9%), 43(43.9%) and 6(6.12%) which were statistically (p=0.026), (p=0.268), (p=0.006) and (p=0.007) respectively, as seen in Table 2. In distribution of duration of exposure to cement dust, from 1 to 10 years, 11 to 20 years and 21 to 30 years groups, asbestos bodies were observed in 7(7.8%), 21(15.8%) and 31(21.1%) of the cases, respectively, which was statistically significant (P=0.000), as seen in Table 3. In distribution of distance of residence to the factory, less than three kilometers and more than three kilometers groups, asbestos bodies were observed among 57(14.8%) and 2(4.3%) of the cases, respectively, which was statistically significant (P=0.029), as seen in Table 4. In distribution of gender, male and female groups, asbestos bodies were observed among 29(10.4%) and 30(19.4%) of the cases, respectively, which was statistically significant (P=0.008), as seen in Table 5. In distribution of residence, Alhagz village, Amran city and factory house groups, high proportion of asbestos bodies were demonstrated in 46(18.6%), 8(6.9%) and 5(7.1%) of the cases, respectively, which was statistically significant (P=0.002), as seen in Table 6. In distribution of the workers and the surrounding population groups, asbestos body were observed among 18(8.6%) and 41(18.4%) of the cases, respectively, which was statistically significant (P=0.002), as seen in Table 7. In distribution of factory departments, casing, preparing and management groups, high proportions of asbestos bodies were observed in 8(10.7%) of the casing workers, as seen in Table 8.

Table 1: Distribution of the study population case control by asbestos bodies.

Category	Cases (Exposed)		Controls		p-value
	Present	Absent	Present	Absent	
Asbestos bodies	59	374	0	98	0.000
	13.6%	86.4%	0%	100%	

Table 2: Distribution of the study population by asbestos cases and cytological changes.

Variable		Cases		Control (N=98)	Total	p-value
		Asbestos cases (N=59)	Non asbestos cases (N=374)			
Cytological atypia	Dysplasia	5(8.5%)	16(4.3%)	0(0%)	21(4%)	0.026
	Metaplastic cells	34(57.6%)	202(54%)	45(45.9%)	281(52.9%)	0.268
Polymorph cells (acute inflammatory)		41(69.5%)	211(56.4%)	43(43.9%)	295(55.6%)	0.006
Macrophage cells (chronic inflammatory)		13(22%)	32(8.6%)	6(6.12%)	51(9.6%)	0.007
Total					531	

Table 3: Distribution of the study population by duration of exposure and asbestos bodies.

Category		1 – 10 years (N=153)	11 – 20 years (N=133)	21 – 30 years (N=147)	p-value
Asbestos bodies	Present	7(4.6%)	21(15.8%)	31(21.1%)	0.000
	Absent	146(95.4%)	112(84.2%)	116(79.1%)	

Table 4: Distribution of the study population by distance of residence and asbestos bodies.

Category		<3km (N=386)	>3km (N=47)	p-value
Asbestos bodies	Present	57(14.8%)	2(4.3%)	0.029
	Absent	329(85.2%)	45(95.7%)	

Table 5: Distribution of the study population by gender and asbestos bodies.

Category		Male(N=278)	Female(N=155)	p-value
Asbestos bodies	Present	29(10.4%)	30(19.4%)	0.008
	Absent	249(89.6%)	125(81.6%)	

Table 6: Distribution of the study population by residence and Asbestos bodies.

Category		Alhagz (N=247)	Amran (N=116)	Factory-house (N=70)	p-value
Asbestos bodies	Present	46(18.6%)	8(6.9%)	5(7.1%)	0.002
	Absent	201(81.4%)	108(93.1%)	65(92.9%)	

Table 7: Distribution of the study population by workers, surrounding population and asbestos bodies.

Category		Worker (N=210)	Surrounding (N=223)	p-value
Asbestos bodies	Present	18(8.6%)	41(18.4%)	0.002
	Absent	192(91.4%)	182(81.6%)	

Table 8: Distribution of the study population by department and asbestos bodies.

Category		Casing (N=75)	Preparing (N=102)	Management (N=33)	P-value
Asbestos body	Present	8(10.7%)	8(7.8%)	2(6.1%)	0.686
	Absent	67(%)	94(%)	31(%)	

DISCUSSION

Industrial pollution and inhalation of mineral dust pose high threat to human health, particularly those residing close to factories and quarries. In this study AsBs were detected in the sputum of 59 (13%) of people exposed to cement dust pollution of whom 18 were cement factory workers. It is interesting to note that frequency of AsBs in sputum was higher in subject living in the surroundings of cement factory compared to factory workers who are supposed to be more exposed. This can be conceived if preventive safety measured (i.e. air filtering, efficient ventilation and protective masks) are applied in the factory. The higher frequency of AsBs in females compared to males could be explained on gender susceptibility. Presence of asbestos bodies in sputum may be an index of lung asbestos burden (Teschler *et al*., 1996). Subject with high asbestos load are greater risk of developing cancer (Koh *et al*., 2011; Giordano *et al*., 2012). In a steam –electric generating plant 3.1% of workers (N = 521) had asbestos bodies in sputum and it was concluded that asbestos bodies counts may be more useful than searching pleural abnormalities by radiograms (Scansetti *et al*., 1993).

In general, the percentage of AsBs positive cases reported here is considered low compared to other reports. Asbestos bodies were found in sputum of about third asbestos workers (Modin *et al*., 1982; Scansetti *et al*., 1993; Scansetti *et al*., 1996) and higher values of 44 % (Paris et al., 2002), 56% (Capellaro et al., 1997) and 75 % (Sebastien et al., 1988) have been observed. In the present study, cytological changes were observed in cement dust exposed cases, positive and negative for AsBs, and in unexposed controls. This suggests that the cytological changes in sputum are not necessarily associated whit inhalation of mineral dust or presence of AsBS. The metaplasia and presence of polymorphnuclear cells macrophages seen in cement dust exposed subjects may indicate an inflammatory state which may be caused one or more of many factors. Sputum cytology changes in asbestos cement workers have been previously observed. Huuskonen *et al*. (1978) reported dysplasia, squamous metaplasia, suspected carcinoma cells foreign bodies in 114 cement workers. Greenberg *et al*. (1976) observed mild to sever atypia, squamous metaplasia and squamous carcinoma in 554 former asbestos cement workers. Setta at al. (2008) and Fell *et al*. (2010) reported high macrophage/ neutrophil ratio and increased number of neutrophils lymphocytes respectively, in asbestos cement workers. In Sudan, mohammad (2008) found sqamous metaplasia and inflammatory cells in non smoker cement workers and Safa and Abdulah (2009) noticed similar changes in people exposed to cement dust. Squamous metaplasia seems to be the most common change reported by most of the authors. High risk of pulmonary cancer in cement production workers has been confirmed (Giordano *et al*., 2012; Koh *et al*., 2011).

CONCLUSION

Asbestos bodies can be detected in sputum smears of people exposed to cement dust and this is associated with different cytological changes. This may constitute high risk of respiratory system cancer. The asbestos fibers risks are increasing with long-term of exposed and closeness.

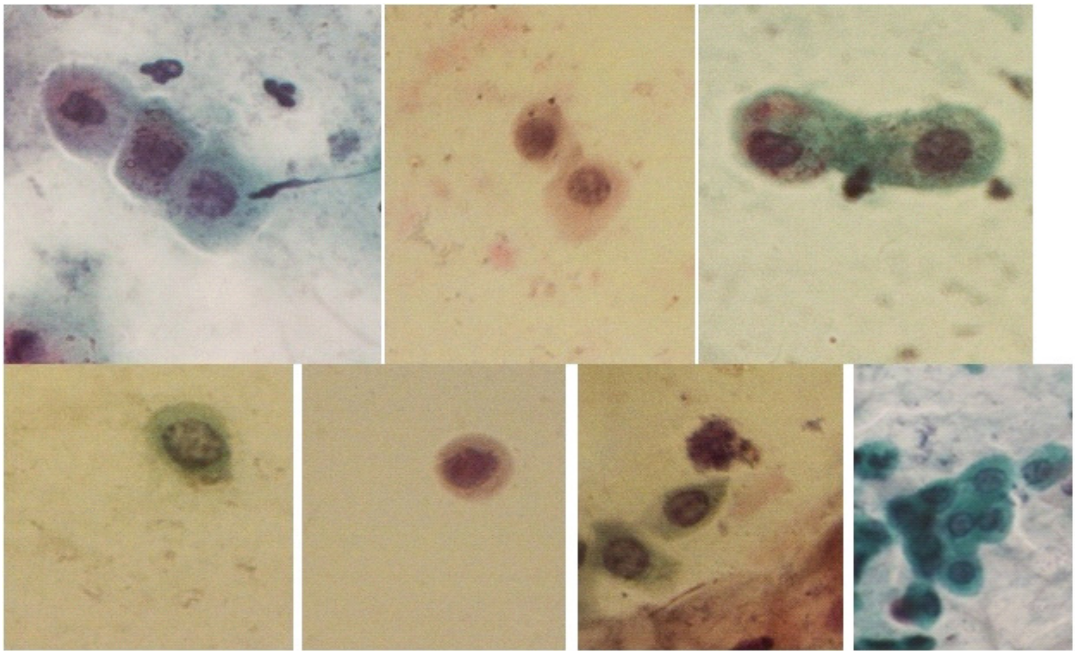

Figure (1): sputum smears from cement dust exposure, showing cytological features of atypical metaplastic cell (Pap stain, x40).

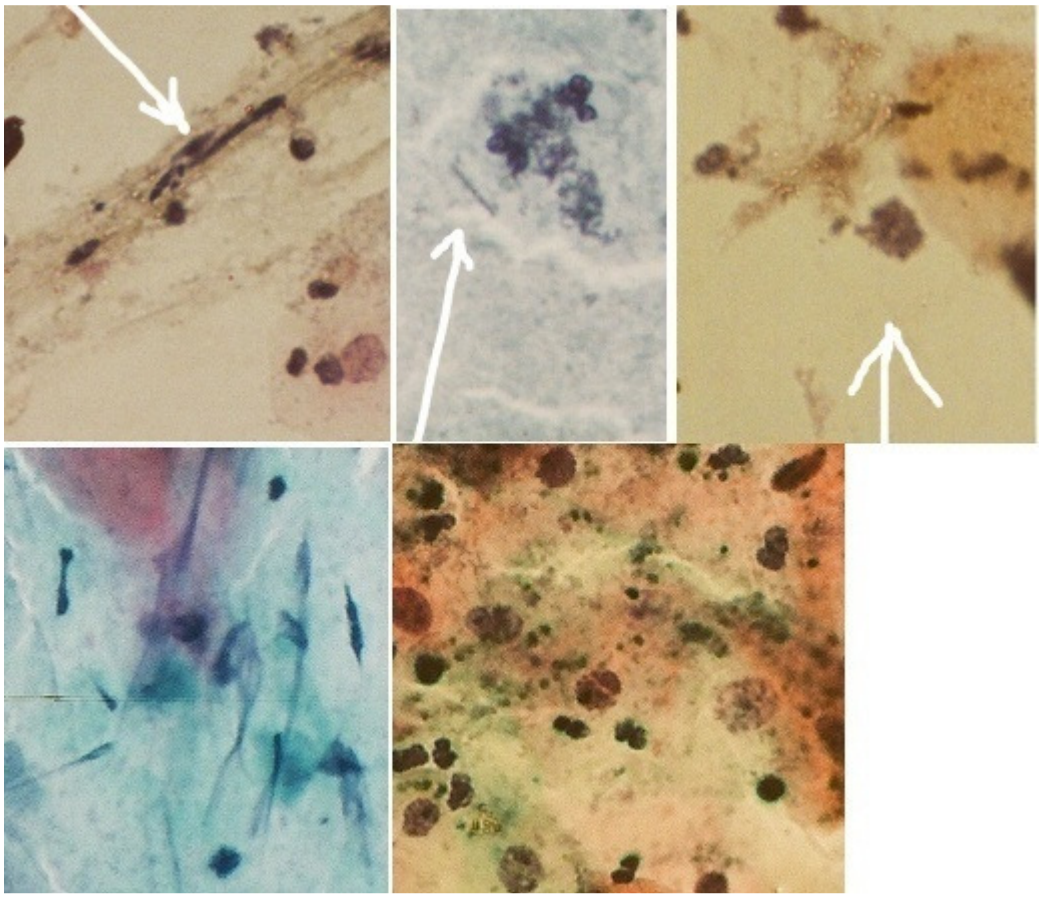

Figure (2): sputum smears from cement dust exposure, showing features of asbestos body and asbestos granules (Pap stain, x40).

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