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Assessment of Lung Epithelial Cytological Changes Associated with Air Pollution in Hail Industrial Area in Saudi Arabia

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ABSTRACT

Objective: *The purpose of this study was to assess the lung epithelial atypical changes (if any), which might be associated with continuous exposure to air pollution resulting from motor vehicles maintenance.* ***Methodology:*** *This is a case control study, investigated 250 participants by cytological methods. Of the 250 participants, 200 were cases exposed to vehicle maintenance emissions air pollution and 50 were controls (non-exposed). Early morning expectorated sputum specimen was obtained and cytologically assessed.* ***Results:*** *Cytological atypia was identified in 7 (3.5%) out of the 200 cases and could not be identified in 193 (96.5%) cases, whereas, in the control group, cytological atypia was not found. The risk of cytological atypia, which is associated with exposure to vehicle maintenance emissions air pollution, the 95% confidence interval (95% CI) and relative risk (RR); RR=3.9 (0.2199 to 69.7010), P=0.3529. Metaplasia was identified among 26 (13%) cases and 2/50 (4%) in the controls. The risk of metaplasia that is associated with exposure to vehicle maintenance emissions air pollution, the 95% CI and RR=3.5 (0.8218 to 15.649), P=0.0893.* ***Conclusion:*** *The exposure to vehicle maintenance air pollution increases the risk of lung epithelial atypical changes, which may develop in to precancerous and cancerous changes.*

Keywords: Lung epithelial atypia, Lung cancer, Air pollution, Sputum, Vehicle emission

INTRODUCTION

Lung cancer continues all over the world as a main cause of death [1]. Lung cancer is a global public health issue and is now the second leading cause of death, following cardiovascular diseases, which it is likely to exceed in the coming years [1,2]. The major lung cancer risk factors are tobacco smoking and environmental air pollution [3,4]. Evidence that air pollution is a risk factor for lung cancer mortality has been reported in a number of case-control studies [5-7]. Although, tobacco smoking is responsible of over 90% of lung cancers [8], but air pollution is established as a major elevator for risk of lung cancer in polluted areas [9]. Several occupational carcinogens, including metals, and polycyclic aromatic hydrocarbons have been confirmed to increase the risk for lung cancer [10,11]. Various work settings could have exposed workers to carcinogens, leading to a greater risk of lung and other cancers [12]. Inhalation of metal dust or fume can cause granulomatous lung diseases. Inhalation of Iron compounds causes siderosis, pneumoconiosis with little or no fibrosis [13]. Motor vehicles and other mobile air pollution causes, such as trucks, trains, buses, and factories have been associated with the cancer risk [14]. Sputum cytology investigation is considered as an essential approach for prediction and detection of lung cancer, since the method is non-invasive and simple to perform. However, cytotyping accuracy in comparison with histologic classification was very high [15,16].

Hail industrial area is a huge area with intensive motor vehicles maintenance and many people working without enough safety precautions. Therefore, the aim of the present study was to assess the lung epithelial atypical changes (if any), which might be associated with continuous exposure to air pollution resulting from motor vehicles maintenance.

PATIENTS AND METHODS

In this case-control study, 250 volunteers living in the city of Hail, northern region of Saudi Arabia were randomly (blinded) selected for the present study and asked to take two days consecutive sputum specimen. Of the 250 participants, 200 were motor vehicles maintenance workers (ascertained as the case group) and 50 were individuals working indoors distant from Hail industrial area (ascertained as the control group). All of the study subjects were men and apparently healthy. Tobacco smokers were excluded from the study population.

Specimen Collection

Sputum specimens were taken from each participant for two consecutive days. For the 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. 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.

Specimen Processing

The smears were stained using the Papanicolaou staining method. 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 hematoxylin 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 rinsed 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 dipped in xylene and mounted in DPX (Distrene Polystyrene Xylene) mount. All the reagents used were from Thermo Electron Corporation, UK.

For assessment of morphological changes and the degree of the quality of the staining, each smear was assessed for quality sufficiency and cellular adequacy. Only stained smears that showed fair staining quality were assessed. For cytological assessment, each slide was assessed for its level of preservation and degeneration by two cytology screeners in a blinded fashion. To reduce the bias, the smears were re-indexed in such a way so that the examiner did not know the cases and controls.

Assessment of Cytological Atypia

Cytological atypia was assessed adopting criteria described by Ahmed, et al. [17]. The presence of two or more of the following features indicated the presence of epithelial atypia: nuclear enlargement associated with increased nuclear cytoplasmic ratio, hyperchromatism, chromatin clumping with moderately prominent nucleation and irregular nuclear borders, bi- or multi-nucleation, increased keratinization and scantiness of the cytoplasm, and variations in size and/or shape of the cells and nuclei. Moreover, the degree of epithelial atypia was assigned by adopting criteria described elsewhere [18].

Data Analysis

SPSS version 16 statistical software was used for statistical analysis. The 95% confidence level was used. Chi-square test was used to compare the differences in categorical variables between the groups. Relationships between variables were analyzed using Friedman's non-parametric test. $P<0.05$ was considered statistically significant.

Informed Consent

Each participant was asked to sign a written informed consent form during the interview, before obtaining the sample.

The informed ethical consent form was designed and approved by the College of Applied Medicine, University of Hail, KSA.

RESULTS AND DISCUSSION

The present study investigated 250 volunteers, their ages ranging from 21 to 63 years with a mean age of 32 years. The age distribution between cases and controls was indicated in Table 1. The majority of the cases were at age range 26-30 years followed by 31-35 years and <25 years representing 61, 39 and 32 persons respectively, hence the majority of controls were with age group <25 years followed by 26-30 years constituting 31 and 14 correspondingly, as shown in Figure 1.

Table 1 Distribution of the study subjects by age and duration of exposure

Variable	Category	Cases	Controls	Total
Age groups	<25 years	32	31	63
	26-30	61	14	75
	31-35	39	4	43
	36-40	26	0	26
	41-45	17	1	18
	46+	25	0	25
Total		200	50	250
Duration of Exposure	<5 years	99	0	99
	6-10	47	0	47
	11-15	30	0	30
	16-20	16	0	16
	21+	8	0	8
Total		200	0	200

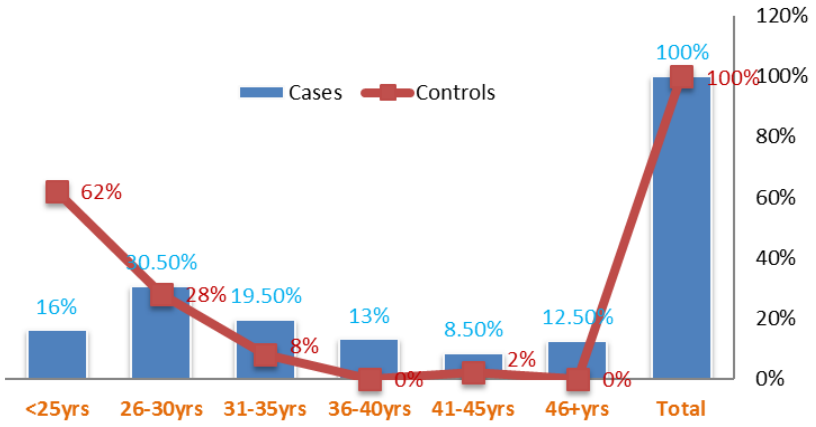

Figure 1 Description of the study population by age

With regard to the duration of exposure to air pollution in industrial area, most of the participants exposed for a duration of <5 years followed by 6-10 years, 11-15 years and 16-20 years representing 99, 47, 30 and 16 persons, respectively as indicated in Table 1.

For distribution of cases and controls by cytological findings, cytological atypia was identified in 7/200 (3.5%) of the cases and could not be found among controls. The risk of cytological atypia that associated with exposure to vehicle maintenance emissions air pollution, the 95% confidence interval (95% CI) and relative risk (RR); RR=3.9(0.2199 to 69.7010), P=0.3529. Metaplasia was identified among 26/200 (13%) of the cases and 2/50 (4%) of the controls. The risk of metaplasia that associated with exposure to vehicle maintenance emissions air pollution, the 95%CI and RR=3.5 (0.8218 to 15.649), P=0.0893.

Acute inflammatory cells infiltrate was identified among 16/200 (8%) of the cases and 10/50(20%) of the controls.

The reverse risk of acute inflammation that associated with exposure to vehicle maintenance emissions air pollution, the 95%CI and RR=0.35 (0.1471 - 0.8227), P=0.0162. Chronic inflammatory cells infiltrate was identified among 36/200(18%) of the cases and 3/50(6%) of the controls. The risk of chronic inflammation that associated with exposure to vehicle maintenance emissions air pollution, the 95%CI and RR=3.4 (1.0136-11.6677), P=0.0475. Cytological evidence of viral infection was identified among 6/200 (3%) of the cases and 1/50 (2%) of the controls. The risk of chronic inflammation that associated with exposure to vehicle maintenance emissions air pollution, the 95% CI and RR=1.5 (0.1783-12.8820), P=0.7034. Fungal infection was identified among 6/200 (3%) of the cases and 0/50(0%) of the controls. The risk of fungal infection that associated with exposure to vehicle maintenance emissions air pollution, the 95%CI and RR=3.4(0.1870 - 60.9219), P=0.4099, as indicated in Table 2 and Figure 2.

Table 2 Distribution of the study subjects by cytological findings

Variable	Cases		Controls		RR (95% CI)	P-value
	Yes	No	Yes	No		
Atypia	7	193	0	50	3.5(0.8218 - 15.649)	0.0893
Metaplasia	26	174	2	48	3.5(0.8218 - 15.649)	0.0893
Acute inflammation	16	184	10	40	0.35(0.1471 - 0.8227)	0.0162
Chronic inflammation	36	164	3	47	3.4(1.0136 - 11.6677)	0.0475
Virus infection	6	194	1	49	1.5(0.1783 - 12.8820)	0.7034
Fungal infection	6	194	0	50	3.4(0.1870 - 60.9219)	0.4099

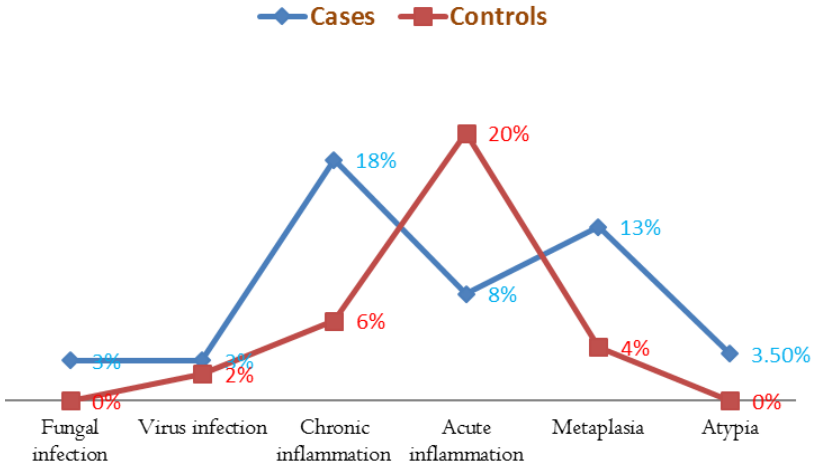

Figure 2 Description of the study subjects by cytological findings

With regard to the age and cytological findings, most cytological atypias were identified among elder individuals 46+ years constituting 4/7 (57.14%). The distribution of metaplasia was relatively similar among different age ranges as indicated in Table 3. Acute inflammatory cells infiltrate was more common among younger age groups (Table 3). Chronic inflammatory cells infiltrate was relatively similar in all age ranges (Table 3). Most cytological evidences of viral infections were found among age group 26-30 years followed by age group 46+ constituting 3/6 (50%) and 2/6 (33.3%) in this order. Most fungal infections were identified at age group 46+ representing 2/6 (33.3%), as shown in Table 3 and Figure 3.

Table 3 Distribution of the study subjects by age and cytological finding

Variable	<25 years	26-30	31-35	36-40	41-45	46+	Total
Atypia	0	1	1	1	0	4	7
Metaplasia	6	6	5	3	3	5	28
Acute inflammation	7	6	4	3	2	4	26
Chronic inflammation	7	5	7	6	6	8	39
Virus infection	0	3	1	0	1	2	7
Fungal infection	1	1	1	1	0	2	6

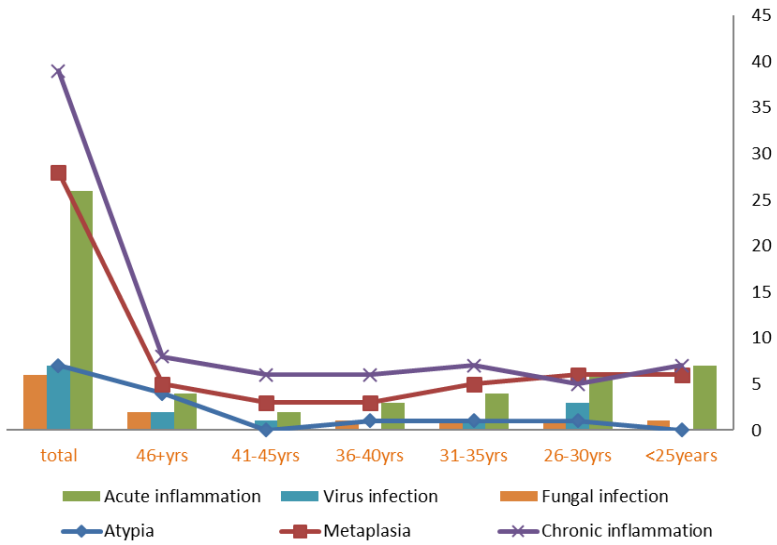

Figure 3 Description of the study subjects by age and cytological findings

Table 4, shows the distribution of the study subjects by duration of exposure and cytological findings; for atypia, most cases of cytological atypia were found among those of 16-20 years of exposure followed by exposure of 21 years or more representing 4/7 (57%) and 2/7 (28.6%), respectively. The frequencies of metaplasia were found to decrease with the increase of duration of exposure to vehicle maintenance emissions air pollution (Table 4). Acute inflammatory cell infiltrate was found to decrease with the increase of duration of exposure (Table 4). Most cases of chronic inflammation were found at duration of exposure 11-15 years, followed by 6-10 years, <5 years and 16-20 years constituting, 17/39 (43.6%), 9/39 (23%), 7/39 (18%) and 5/39 (12.8%), respectively. Most viral and fungal infections were found at duration of 6-10 years, as indicated in Table 4 and Figure 4.

Table 4 Distribution of the study subjects by duration of exposure and cytological findings

Variable	<5years	06-10	11-15	16-20	21+	total
Atypia	0	1	0	4	2	7
Metaplasia	9	9	5	3	2	28
Acute inflammation	14	7	3	2	0	26
Chronic inflammation	7	9	17	5	1	39
Virus infection	1	2	2	1	1	7
Fungal infection	1	3	1	0	1	6

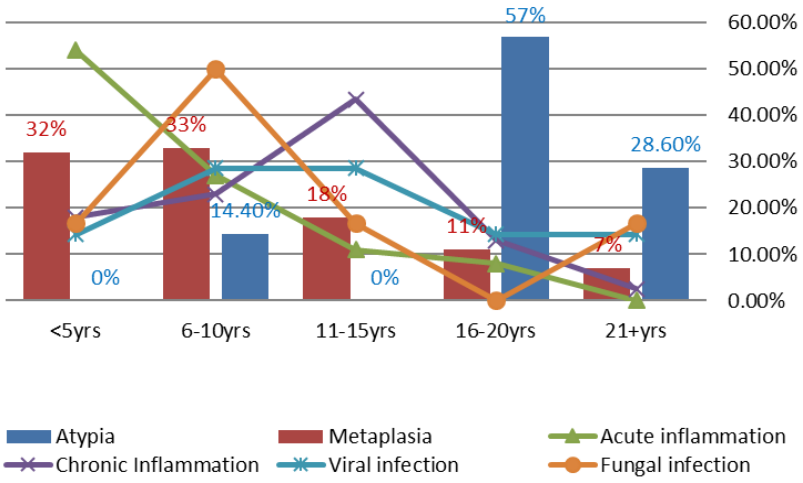

Figure 4 Description of the study subjects by duration of exposure and cytological findings

DISCUSSION

In recent years Saudi Arabia has witnessed rapid development with fast growth of cities and population, which speeded up the need for increased transportation facilities [19]. This results in a huge usage of motor vehicles, which required large industrial areas for their mechanical maintenance. Hail industrial area is a huge area with intense mechanical maintenance with intense emission of environmental pollutants resulting from these motor vehicles maintenance.

Therefore, our aim was to assess the lung epithelial atypical changes, which might be associated with prolonged exposure to air pollution resulting from motor vehicles maintenance in Hail region of Saudi Arabia. Environmental air pollution has become a leading health concern especially in the developing countries with more urbanization, industrialization and rapidly growing population. Prolonged exposure to air pollution is a risk factor for many respiratory diseases including lung cancer [20,21].

In this study lung epithelial proliferative activity in form of cytological atypia and metaplasia was encountered in many cases. Such findings were previously indicated in several studies. The International Agency for Research on Cancer (IARC) has classified diesel engine exhaust as carcinogenic to humans (Group 1) [22]. Motor vehicle emissions account for 25-40% of air pollution [23]. Vehicle emissions are producing gaseous and particulate pollutants such as, carbon monoxide, ozone, particulate matter, nitrogen dioxide aldehydes, benzene, 1,3-butadiene, polycyclic aromatic hydrocarbons, and metals [23]. Meta-analysis study found that ambient exposure to nitrogen oxides, sulfur dioxide, and fine particulate matters significantly increase the risk of lung cancer. Most ambient nitrogen dioxide is resulting from oxidation of nitrogen monoxide, which is principally made by vehicle emissions [24]. Furthermore, it was reported that, occupational exposure to air pollution among professional drivers significantly increased the incidence and mortality of lung cancer [21].

As sputum cytology is simple, cheap, and non-invasive with relatively reasonable specificity and sensitivity, can applied for screening and detection of early lung epithelium proliferative activity, which might subsequently, develops into lung cancer. In the present study, though the presence of lung epithelial atypia and metaplasia among cases were statistically insignificant, the risks associated with exposure were relatively high. Such findings were previously reported in similar settings [7,12]. It was found that the inhalation of sulfur dioxide produces multi-organ DNA lesions, including in the lung, which can progress into mutation, cancers, and related disorders [25]. There are variable mechanisms encountered in the cellular response to air pollution. Several studies have shown that prolonged exposure to air pollutants can yield substantial alterations in gene expression patterns [26,27], and in human alterations in transcriptional profiles have been associated with air pollution exposure [28-30].

With regard to the duration of exposure to air pollutants, most cases of cytological atypia and metaplasia occurred relatively after prolonged period of exposure. Moreover, it was witnessed that during specimen collection, all of the vehicle maintenance workers were non-Saudi and less educated. They ignore all safety measures, such as wearing masks, which are useful tools to reduce their exposure. Such people need educational awareness programs towards long-lasting air pollution hazards and on how they can reduce them.

In the current study, the acute inflammatory cells infiltrate was insignificant, but the chronic inflammatory cells infiltrate was statistically significant among cases. However, the presence of chronic inflammatory cells in the sputum of the cases may guess continuous inflammatory mechanisms resulting to continuous air pollutants exposure. Therefore, more studies are needed in this area with an association with what is known as the neutrophil-to-lymphocyte ratio (NLR). The NLR is an inflammatory index that has been deliberated as a prospective prognostic factor in human cancer [31]. Furthermore, it was observed that in this study, the majority of abnormal variables were found among cases compared to controls regardless to the number of participants in each group. This may verify the harmful effects of vehicle emission occupational exposure on the epithelium of respiratory tract, which might ultimately result in the development of precancerous and cancerous lesions.

Although this has notified for the use of sputum cytology as simple screening method for population at risk, but it has some limitations, such as the use of more advancer confirmatory techniques (histopathology or molecular methods). On the other hand, this study upraised some controversies for policy makers to plan strategies for assessment and control of vehicle emission related air pollution in northern Saudi Arabia. This, in addition to the fact that, the study may stimulate further research in this concern.

CONCLUSION

The exposure to vehicle maintenance air pollution increases the risk of lung epithelial atypical changes, which may develop to precancerous and cancerous changes. Sputum cytology may be a reliable non-invasive and cost-effective method for screening individuals exposed to vehicle emission related air pollution. Awareness programs for vehicles maintenance workers are necessary, mainly for wearing masks.

DECLARATIONS

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Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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