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Research Article

Lung Epithelial Atypia associated with Automotive Maintenance Mechanics Related Air Pollution in Hail, North Saudi Arabia

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Abstract: The association between lung cancer and smoking was well established, but the link between lung cancer and automotive maintenance mechanics air pollution still uncertain. To define the link between automotive maintenance mechanics and lung epithelial atypical changes, we assessed using cytological method; cytological changes in sputum specimens. Methods: Sputum samples were collected from 150 apparently healthy volunteers, living in the city of Hail north of Saudi Arabia. Of the 150 participants, 100 were workers in automotive maintenance mechanics in the industrial area (ascertained as Cases) and 50 were non-exposed (ascertained as Controls).Results: Cytological atypia was detected in 2/100(2%)of the cases and none in the controls. The adjusted Odd Ratio (OR) and the 95% confidence interval was found to be 2.5635 (0.1208 to 54.4163), P value = 0.5459. Metaplasia was identified among 9/100 (9%) of the cases and 4/50(8%) of the controls. For the metaplasia among cases, the adjusted OR and the 95% confidence interval was found to be 0.75(0.2517 to 2.2351), P value = 0.517. The risk of atypia and metaplasia was found to increase with increase of exposure and these were found to be statistically significant P value < 0.009, respectively. Conclusion: Exposure to automotive maintenance mechanics was associated with risks for developing dysplasia and high risks of development of metaplasia. Sputum cytology may provide a suitable technique for the assessment of lung epithelial atypical changes.
Keywords: Lung epithelial, automotive maintenance mechanics, air pollution, Saudi Arabia

INTRODUCTION

Lung cancer has been a major health problem in developed countries for several decades, and has emerged recently as the leading cause of cancer death in many developing countries [1]. Lung cancer is the most common cause of cancer mortality worldwide for both men and women, causing approximately 1.2 million deaths per year [2]. Lung cancer accounts for 4% of all newly diagnosed cancers in Saudi Arabia. Squamous cell carcinoma was the most common cell type, and significantly associated with smoking. The incidence of metastasis was high at presentation. The right lung and right upper bronchus were often affected. Hypercalcemia and hyponatremia were the most common biochemical abnormalities [3]. Exposure to genotoxic carcinogens in tobacco smoke is a major cause of lung cancer. However, the effect this has on DNA copy number and genomic stability during lung carcinogenesis is unclear [4]. A few prospective studies demonstrated that bilirubin levels were inversely associated with the risk of lung cancer. Lower levels of bilirubin may be a risk factor for lung cancer [5]. Some of the most important lung cancerdrivers are mutations in the EGFR gene [6]. Macrophages were shown to play significant roles in regulating inflammation, oxidative stress, and viral infection following alcohol exposure in the liver, lungs, adipose tissue, and brain [7]. Smoking is the primary cause of lung cancer, as well as, and alcohol consumption contributes to lung cancer risk [8]. The level of outdoor and indoor air pollution resulting from industrial and motor vehicle emissions has been increasing at an accelerated rate. Thus, there is a significant increase in the prevalence of respiratory symptoms such as coughing, wheezing, and decreased pulmonary function. Experimental exposure research and epidemiological studies have indicated that exposure to particulate matter, ozone, nitrogen dioxide, and environmental tobacco smoke have a harmful influence on development of respiratory diseases and are significantly associated with cough and wheeze [9]. Exposure to air pollution is associated with increased morbidity from cardiovascular diseases, lung cancer, respiratory and allergic diseases [10]. Air pollution causes lung cancer [11]. Air pollution is the primary environmental stressor in relation to cardiovascular morbidity and mortality [12]. Lung cancer is usually

suspected in individuals who have an abnormal chest radiograph finding or have symptoms caused by either local or systemic effects of the tumor. The method of diagnosis of suspected lung cancer depends on the type of lung cancer[13]. Sputum cytology is an acceptable method of establishing the diagnosis of lung cancer, the sensitivity of sputum cytology varies according to the location of the lung cancer [14]. Several different testing methods offer a more sensitive alternative to direct sequencing for the detection of common mutations. Evidence published to date suggests cytology samples are viable alternatives for mutation testing when tumor tissue samples are not available [15].

Most studies on long-term exposure to air pollution and lung cancer risk have investigated the association with lung cancer mortality. However, in this, we assessed using cytological method lung epithelial atypia to determine the relationship between exposure to automotive maintenance mechanics air pollution and lung epithelial atypia in Saudi Arabia, Hail.

MATERIALS AND METHODS

Study Design

The Retrospective Cohort has been described in detail [16]. Briefly, this Saudi Retrospective Cohort Study on automotive maintenance mechanics was started in September 2014 with the enrollment of 250 apparently healthy volunteers (200 worker in automotive maintenance mechanics "exposed" and 50 indoors employees "non-exposed") aged 25 to 56 years living in the city of Hail, Saudi Arabia. The study was designed as a case/control-cohort study, ie, cases were derived from the workers in automotive maintenance mechanics at the industrial area for at least 5 hours per day, whereas the person-years at risk were estimated from a random sub-cohort (N = 3000); controls were derived from individuals working in closed offices for at least 5 hours per day. This approach was chosen for efficient processing of the baseline questionnaire. Approximately 100% of workers in automotive maintenance mechanicsin the Saudi are men, and therefore, all study subjects in this study were men. Details of the exposure assessment have been described according to long-term exposure to industry area in the city of Hail, Saudi Arabia. The sample size was calculated from the total number of the works in automotive maintenance mechanic in the city of Hail. On the other hand, the sample size for control was set as 50, without referring to a specific equation for calculation, and they represented the apparently healthy indoors employees.

Sputum specimens were taken from each participant for three 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 smears were fixed immediately in 95% ethyl alcohol while it was wet.

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 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) mount.

All the reagents used were from Thermo Electron Corporation, UK.

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.

Statistical Analysis

Automotive maintenance mechanics related Air Pollution effects were analyzed for overall exposure with other variables to identify effects on the lung epithelial cells. Odd Ratio (OR) 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 and P value less than 0.05 were considered statistically significant.

Ethical Consent

The study was approved from Department of Clinical Laboratory Science, College of Applied Medical Science, University of Hail, and Kingdom of Saudi Arabia. All study subjects consented for their participation by completing the self-administered questionnaire.

RESULTS

This study investigated 150 males, their ages ranging from 21 to 63years old with mean age of 32 years. The age distribution was relatively differing among the cases and the controls. The vast majority of the controls were belonged to the age group > 25 years representing 31/50 (62%), since most cases were found in age range 26-35 years' constituted 57/100(57%). Atypia (Dysplasia) was detected in 2/100(2%) of the cases and none in the controls. For the atypia among cases, the adjusted OR and the 95% confidence interval was found to be 2.5635 (0.1208 to 54.4163), P value = 0.5459. All of cases of atypia were identified among those who worked for durations of more than 17 years. This indicates that the risk of atypia increase with increase of exposure and this was found to be statistically significant P value < 0.0001.

Metaplasia was identified among 9/100 (9%) of the cases and 4/50(8%) of the controls. For the metaplasia among cases, the adjusted OR and the 95% confidence interval was found to be 0.75 (0.2517 to 2.2351), P value = 0.517. The risk of metaplasia was also fount to increase with increase of exposure and this was found to be statistically significant P value < 0.009.Acute inflammatory cells infiltrates were found in 6(6%) of the cases and 10(20%) of the controls. Acute inflammation was reversely associated with exposure to automotive mechanic the adjusted OR and the 95% confidence interval was found to be 0.2553 (0.0869 to 0.7501), P value = 0.0130. Chronic inflammatory cells infiltrates were found in 15(15%) of the cases and 6(12%) of the controls. For the chronic inflammatory cells infiltrates among cases, the adjusted OR and the 95% confidence interval was found to be 1.2941(0.4693 to 3.5686)P = 0.6183.

In regard the relationship between age and cytological findings, cytological changes was found to increase with the increase of age and this was found to be statistically significant P < 0.004, as indicated in Table 2, Fig 2.

Table 1. Distribution of the study population by sputum cytological findings

Variable	Cases		Controls		P value
	Present	Absent	Present	Absent	
Atypia	2	98	0	50	0.54
Metaplasia	9	91	4	46	0.75
Acute inflammatory cells	6	94	10	40	
Chronic inflammatory cells	15	85	6	44	0.61

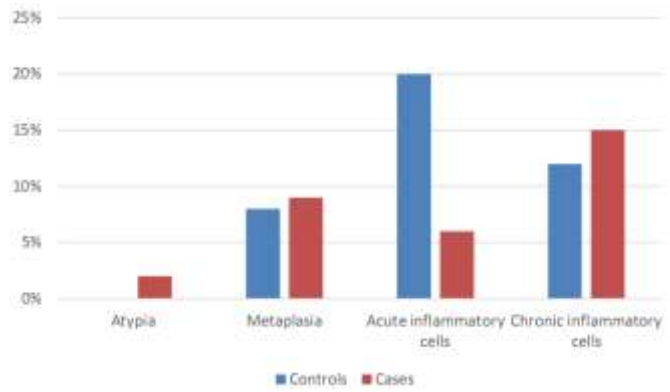

Fig. 1. Description of the study population by the proportions of cytological findings

Table 2. Distribution of the study subjects by age and cytological changes

Variable	Category	Age range				Total
		<25	26-35	36-45	46+	
Atypia	Yes	0	0	0	2	2
	No	20	57	19	2	98
Total		20	57	19	4	100
Metaplasia	Yes	1	4	5	3	13
	No	19	53	14	1	87
Total		20	57	19	4	100

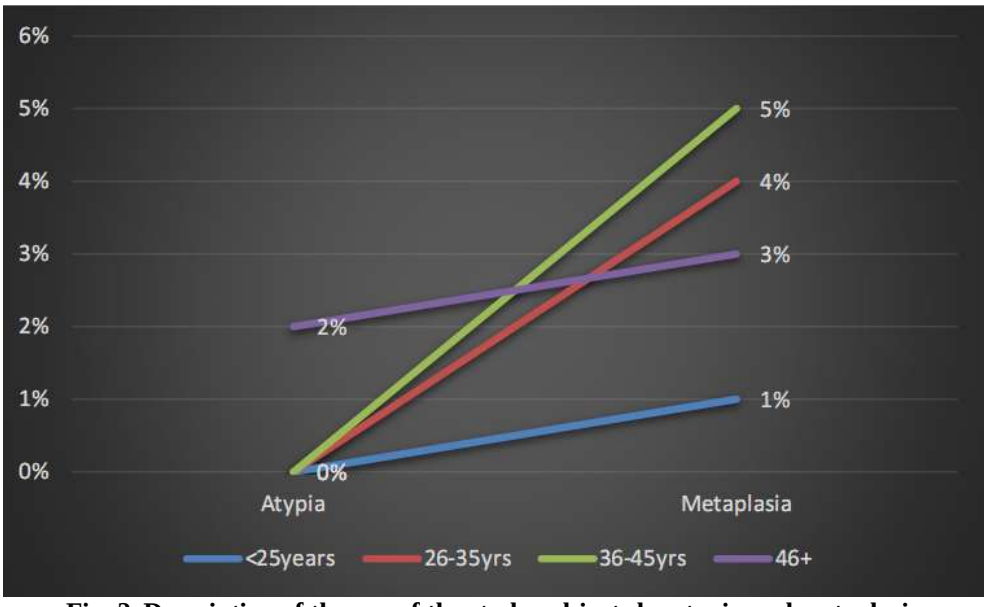

Fig. 2. Description of the age of the study subjects by atypia and metaplasia

DISCUSSION

To evaluate the impact of environmental automotive maintenance mechanics related Air Pollution on lung cancer risk, this study examined by cytological methods two different occupational groups. The first group was automotive maintenance mechanics (exposed for at least 5 hours per day (ascertained as cases group), and a second group comprised of individuals working in closed offices for at least 5 hours per day (non-exposed (ascertained as control group)).

In the present study strong associations between automotive maintenance mechanics related air pollution and lung epithelial changes have been observed in cases, as well as,the relative risk increases with the extent of the duration of exposure.

Dysplasia (cytological atypia) which is believed to be precursors of squamous cell carcinoma [17] was demonstrated in seven (2%) of the cases and none of the controls. It is well known that there is close association between the occupational and/or environmental causes of cancer and prevalence of cancers. The International Agency for Research on Cancer (IARC) identified 415 known or suspected carcinogens. Cancer ascends through an exceedingly complicated set of various causes as single agents or combinations of agents. It is well known that preventing exposure to individual carcinogens prevents the disease. Declines in cancer rates-such as the drop in male lung cancer cases from the reduction in tobacco smoking or the drop in bladder cancer among cohorts of dye workers from the elimination of exposure to specific aromatic amines-provides evidence that preventing cancer is possible when there is suitable intervention[18].

Surprisingly there is a complete absence of data regarding the long-term health hazards of automotive maintenance mechanics related air pollution, including its impact on the development of lung cancer. Several studies on the association between lung cancer and air pollution have studied mortality [19]. To determine what, if any, additive risk was existing by ambient air pollution, we focused on automotive maintenance mechanics workers, as they are constantly exposed to high concentrations of air pollutants with prolonged durations. Motor vehicles and other mobile air pollution sources, such as trucks, trains, buses and factories have been linked to the cancer risk [20].

Metaplasia was identified among 9%. Metaplasia is the reversible replacement of one differentiated cell type with another mature differentiated cell type, which is generally caused by some sort of abnormal stimulus. If the stimulus that produced metaplasia is detached or ceases, tissues return to their normal form of differentiation. Metaplasia is not synonymous with dysplasia and is not directly considered premalignant but it can progress in to premalignant [21,22]. However, exposure to diverse air pollutants was connected to epithelium metaplasia in the respiratory tract [23]. Diesel exhaust particles prohibited serum starvation-led declines in epithelial cells by bringing cell cycle progression and preventing apoptosis processes including oxidative stress, inhibition of expression and stimulation of N-terminal kinase and nuclear factor-B. Consequently, low-dose diesel exhaust particle exposure may lead to lung epithelialcell hyperplasia. With the increasing use of diesel-powered engines, particulate air pollution is increasingly being recognized as a major public health hazard and as a contributor to the burden of lung diseases. In addition, a relationship between mortality and lung metaplasia in adults living in metropolitan areas and the level of particulate air pollution has been reported [21,23].

Moreover, inflammatory cells infiltrates were detected among the whole studied subjects, but more frequent among cases than controls. On exposure to air pollutants airway epithelial cells, which form the first line of innate immune defense against particles, produce inflammatory response [23]. In addition, air pollutants can also cause cell death by necrosis, and by the process of apoptosis, which can increase inflammatory potency [24]. Some studies have stated that exposure to some air pollutants potentiates acute inflammation and mucus production and secretion elicited by a biogenic substance in rat pulmonary airways [25]. Moreover, the presence of viral or fungal which may be due to deteriorated immunity can contribute to the presence of inflammatory cells infiltrates.

CONCLUSION

This study established an association between exposures to automotive maintenance mechanics related air pollution and lung dysplasia. Exposure to automotive maintenance mechanics related air pollution was also connected to a raised risk for development of metaplasia. Preventive measures are highly recommended in Hail industrial area.

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