



The difference of nasal mucosal cytology features in gas station workers compared to non-gas station workers

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ABSTRACT

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Petroleum hydrocarbons such as benzene, toluene, ethylbenzene and xylene (BTEX) are the most common pollutants that cause environmental pollution and has harmful effects on the human mucosal membrane irritation. This cross-sectional design study aimed to evaluate the difference in cytological nasal mucosa between gas station workers and non-gas station workers. There were 80 subjects with inclusion criteria i.e. aged 20-50 years, worked more than 6 months, consisting of 40 gas station workers (exposed group) and 40 non-gas station workers (unexposed group). The exclusion criteria of both groups were use nasal drops in the last ten days, acute rhinitis, had nasal trauma, had nasal surgery, consume alcohol, history of allergic rhinitis, and refused to participate in the study. All subjects performed brushing at 1/3 anterior nose inferior turbinate and cytology examination. The statistical analyses used Chi-square tests. From the exposed group we found 18 (45%) subjects with inflammation, 17 (42.5%) with metaplasia, and 9 (22.5%) with dysplasia, while in the unexposed group there were 10 (20.5%) subjects with inflammation, 4 (10.0%) with metaplasia and 2 (5.0%) dysplasia. There were significantly differences in nasal mucosal cytology, particularly metaplasia ($p = 0.001$; $RP = 6.65$; $95\% CI = 1.78-27.01$) and dysplasia ($p = 0.023$; $RP = 5.52$; $95\% CI = 1.22-32.10$) between both groups. It can be concluded that there are significantly differences involving metaplasia and dysplasia in nasal mucosa cytology features of gas station workers compared to non-gas station workers.

ABSTRAK

Hidrokarbon bahan bakar minyak bumi seperti benzene, toluene, ethylbenzenem, dan xylene (BTEX) adalah polutan paling umum yang menyebabkan pencemaran lingkungan dan memiliki efek berbahaya berupa iritasi membran mukosa manusia. Tujuan penelitian desain cross-sectional ini untuk menentukan perbedaan sitologis mukosa hidung antara pekerja Stasiun Pengisian Bahan Bakar Umum (SPBU) dan bukan pekerja SPBU. Penelitian dilakukan pada 80 sampel dengan kriteria inklusi usia 20-50 tahun, bekerja lebih dari 6 bulan, terdiri atas 40 sampel pekerja SPBU (kelompok terpajan) dan 40 sampel bukan pekerja SPBU (kelompok tidak terpajan). Kriteria eksklusi kedua kelompok adalah tidak menggunakan obat tetes hidung, sedang menderita rhinitis akut, pernah trauma hidung, pernah operasi hidung, alkoholik, menderita rhinitis alergi, dan menolak berpartisipasi dalam riset. Terhadap seluruh sampel dilakukan pemeriksaan brushing pada 1/3 anterior konka nasalis inferior dan pemeriksaan sitologi. Analisis statistik menggunakan uji Chi-kuadrat. Hasil pemeriksaan sitologi didapatkan pada kelompok terpajan didapatkan 18 (45.0%) subjek dengan inflamasi, 17 (42.5%) metaplasia, 9 (22.5%) displasia, sedangkan pada kelompok tidak terpajan didapatkan 10 (25.0%) subjek dengan inflamasi, 4 (10%) metaplasia dan 2 (5%) displasia. Perbedaan metaplasia ini berbeda secara statistik ($p: 0.001$; $RP: 6.65$; $95\% CI: 1.78-27.01$) dan displasia ($p = 0.023$; $RP = 5.52$; $95\% CI = 1.22-32.10$) antara kedua kelompok. Berdasarkan hasil penelitian ini dapat disimpulkan terdapat perbedaan nyata metaplasia dan displasia mukosa hidung antara pekerja SPBU dan bukan pekerja SPBU.

Keywords:
gasoline vapors
nasal mucous
cytology
metaplasia
dysplasia

INTRODUCTION

Monoaromatic hydrocarbons are major components of gasoline and are thought to be the most prevalent contaminants of soil and groundwater due to frequent leakages from underground storage tanks and accidental spills. Petroleum hydrocarbons such as benzene, toluene, ethylbenzene and xylene (BTEX) are one of the most common pollutants that cause environmental pollution.^{1,2} These compounds are volatile monoaromatic hydrocarbons presenting in petroleum, gasoline, and industrial solvents. The BTEX has been introduced in the environment through petrochemical wastewater, fuel leakage from storage tanks and transportation. It has harmful effects on the human health that lead to mucosal membranes irritation, cancer, impairment of the central nervous system, and liver and kidney disruption.^{3,4}

Human exposure to gasoline usually causes side effects on health depending on the route of exposure and quantity of gasoline involved. Various degrees of toxicities are also associated with gasoline inhalation and sometimes anaemia and other disease condition may result.⁵ The practice of aspiration of premium motor spirit (PMS) by mouth was an important source for exposure. Although the primary route of exposure to benzene, a volatile chemical organic compound, is by inhalation of PMS fume.⁶

Xylene irritation of the nose and throat can occur at approximately 200 ppm after 3–5 min. Accidental splash in the eye may damage the surface of the eye, which will heal within a few days. Considering an allowable work time is 8 h a day, Nordic filling station workers are exposed to about 0.5-1 mg/m³ benzene from gasoline vapors at gas stations especially when refueling a car tank. While dispensing a 30 L load containing 5% by volume of benzene

into the car, there is present about 700 mg of inhaled benzene. The total concentration of hydrocarbons in the air when the process of refueling gasoline is 10 to 100 times benzene. Workers at gas stations can also be exposed to vehicle emissions gas, including aromatic polycyclic hydrocarbons, aldehydes, and 1,3-butadiene. The volume of benzene in gasoline fuels ranges from 2-6% in the Nordic countries.⁶ A study was conducted on the incidence of cancer among 19,000 gas station workers from Denmark, Norway, Sweden, and Finland. They were identified since 1970 and followed up after 20 years, and the results showed that there were 1,300 workers suffering from cancer.⁷ According to The International Agency for Research on Cancer (IARC, 1987, 1999, 2000), The United States Environmental Protection Agency (USEPA, 2004) has evaluated materials in BTEX that are potentially as carcinogenic agent.⁸

Until now there is still no awareness of the effects of exposure to gasoline on the nasal mucosa, as well as the effects that can damage the anatomy and function of nasal mucosa. This study aimed to determine the medical evidence of different nasal mucosal cytology features in gas station workers compared to non-gas station workers.

MATERIALS AND METHODS

Study design

This was a cross-sectional study conducted in the Department of Otorhinolaryngology Head and Neck Surgery at Dr. Sardjito General Hospital Yogyakarta between August 2012 until December 2013. Histopathologic examination was conducted at the Anatomic Pathology Department, Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada/Dr. Sardjito General Hospital Yogyakarta Indonesia. The study obtained approval from the Medical and Health Research Ethics

Committee (MHREC) Faculty of Medicine Universitas Gadjah Mada, Yogyakarta Ref: KE/FK/553/EC.

Population and subjects

We included gas station workers aged 20-50 years living in Yogyakarta who were exposed to gasoline vapors and had been working at least three months and did not use nasal drops in the last ten years. All subjects with a history of acute rhinitis, nasal trauma, nasal surgery, alcohol consumption and allergic rhinitis were excluded from the study. The exclusion criteria of both groups were: 1) patients with acute rhinitis, 2) has nasal trauma, 3) has nasal surgery, 4) consume alcohol, 5) history of allergic rhinitis, and 6) refused to participate in the study. All study samples performed anamnesis and underwent physical examination. Sample calculation based on α : 5% and β : 20%, obtained 40 samples for each group.

Examination procedures

All of 80 subjects performed brushing on 1/3 anterior of inferior nasal mucous turbinate by Anatomy Pathology Specialist of The Anatomy Pathology Department the Faculty of Medicine, Universitas Gadjah Mada Yogyakarta to determine the possibility of inflammation, metaplasia, or dysplasia cytologic feature.

Statistical analysis

All statistical analyses were carried out using SPSS for Windows version 24. Participants characteristics including sex, age, length of work, smoking habit, nasal congestion, runny nose, sneezing were analyzed descriptively. Parameters of nasal mucosal cytology including inflammation, metaplasia and dysplasia were treated as categorical variables. The significance test of the difference in proportion between cytologic changes between the two groups was analyzed using Chi-squared test.

RESULTS

There were 80 subjects included in the final analyses. Characteristics of these subjects were shown in TABLE 1. There were 54 (67.5%) males and 26 (32.5%) females. Using Chi-square test, there was no significant difference between the exposed groups and unexposed groups ($p = 0.152$). The age range of the study subjects was 20 to 50 years old. Age was divided into two categories. The largest age frequency was 20-30 year consisted of 25 (43.8%) exposed subjects and 32 (56.2%) unexposed subjects. Based on comparison results using Chi-squared test, there was no significant differences in both age groups of subjects exposed and unexposed ($p = 0.084$).

TABLE 1. Clinical characteristics and complaints of subjects

Variable	Exposure n (%)	No exposure n (%)	Total n (%)	p
Sex				
• Male	30 (75.0)	24 (60.0)	54 (67.5)	0.152
• Female	10 (25.0)	16 (40.0)	26 (32.5)	
Age (year)				
• 20-30	25 (62.5)	32 (80.0)	57 (71.25)	0.084
• 31-50	15 (37.5)	8 (20.0)	23 (28.75)	
Length of work (year)				
• \leq 5 year	22 (55.0)	21 (52.5)	43 (53.75)	0.283
• > 5 year	18 (45.0)	19 (48.5)	37 (46.25)	

Smoking habit				
• Yes	19 (47.5)	0 (0.0)	19 (23.75)	0.001
• No	21 (52.5)	40 (100.0)	61 (66.25)	
Nasal congestion				
• Yes	7 (17.50)	0 (0.0)	7 (8.75)	0.006
• No	33 (82.5)	40 (100.0)	73 (91.25)	
Runny nose				
• Yes	7 (17.50)	0 (0.0)	7 (8.75)	0.006
• No	33 (82.5)	40 (100.0)	73 (91.25)	
Sneezing				
• Yes	7 (17.50)	0 (0.0)	7 (8.75)	0.006
• No	33 (82.5)	40 (100.0)	73 (91.25)	

TABLE 2 shows the outcome of this study involved nasal mucous cytologic features. The frequency of the inflammatory feature in the exposed subjects were 18 (45.0%) subjects and 22 (55.0%) subjects were normal, while

in the unexposed subjects there were 10 (25.0%) samples with inflammation and 30 (75.0%) normal samples. However, this difference was not statistically significant ($p = 0.061$; $RP = 3.516$; $95\% CI = 0.86-7.1$).

TABLE 2. The relationship between gasoline vapors exposure and epithelial changes.

Epithelial changes	Exposure	No exposure	p	Ratio prevalence	95% CI
Inflammation					
• Positive	18 (45.0)	10 (25.0)	0.061	3.516	0.86-7.10
• Negative	22 (55.0)	30 (75.0)			
Metaplasia					
• Positive	17 (42.5)	4 (10.0)	0.001	6.650	1.78-27.01
• Negative	23 (57.5)	36 (90.0)			
Dysplasia					
• Positive	9 (22.5)	2 (5.0)	0.023	5.520	1.22-32.10
• Negative	31 (77.5)	38 (95.0)			

DISCUSSION

In this study, we demonstrated chronic exposure of gasoline to the nasal mucosal cytology features in gas station workers compared to non-gas station workers. Many studies showed the alterations in lung structure of the alveolar parenchyma, associations between air pollution and exacerbations of pre-existing COPD, but the role of air

pollution in the nasal mucosal cytology features in gas station workers is still uncertain.¹¹

Based on the age distribution results in this study are consistent with the study by Udonwa *et al.*⁶ where the largest age distribution was the age of 20-29 years as many as 23 (46%) samples. All characteristic variables from samples in this study were divided into 2 categories except the education level. In this study,

there were statistically significant differences in the characteristic variables of education level and smoking habit. The other variables included nasal obstruction complaints, runny nose and sneezing were not statistically significantly different (TABLE 1).

Diesel exhaust particles (DEP) contain organic and inorganic elements that produce damage to the respiratory epithelium. The explants models reproduce and display various key features of *in vivo* airways, such as mucus coverage, mucociliary clearance, and cell structure, which adds a further dimension in examining the *ex vivo* interactions between epithelia and underlying structural cells of airway mucosa.¹²

This study found the frequency of metaplasia in the exposed group was 17 (42.5%) subjects and 23 (57.5%) normal subjects. In the not exposed group there were 4 (10%) subjects with metaplasia and 36 (90%) normal subjects (TABLE 2). This difference was statistically significant ($p = 0.001$; $RP = 6.65$; 95% $CI = 1.78-27.01$). The exposed group to gasoline vapors had the prevalence of metaplasia 6.65 times greater than that in the not exposed.

The frequency of the dysplasia cytologic feature in the exposed groups were 9 (22.5%) subjects and 31 (77.5%) normal subjects, while in the not exposed groups there were 2 (2.5%) dysplasia subjects and 38 (97.5%) subjects had normal cytologic features. This difference was statistically significant ($p = 0.023$; $RP = 5.52$; 95% $CI = 1.22-32.10$). The exposed groups to gasoline vapors had a prevalence of dysplasia 5.52 times greater than that in the not exposed groups. Another study found also statistically significant differences in nasal mucosal cytology, particularly metaplasia ($p = 0.008$; $OR = 7.857$; 95% $CI = 1.495-41.302$) and dysplasia ($p = 0.018$; $OR = 4.251$; 95% $CI = 1.945-19.123$) between both group subjects.¹³

Short term exposure to mixed xylene or their individual isomers result in irritation of the nose, eyes and throat subsequently leading toward neurological, gastrointestinal and reproductive harmful effects. In addition to long term exposure to xylene may cause hazardous effects on respiratory system, central nervous system, cardiovascular system, and renal system.¹⁴

The deleterious effects of air pollution and petrol/diesel vapor inhalation on the lung function of petrol pump workers results in a restrictive type of lung function abnormality. The pattern of respiratory impairment changes to a mixed type as the duration of exposure increases. the exposure of petrol pump workers to the benzene content of petrol has shown the highest benzene concentrations in the breathing zone of petrol station attendants. The study also shows that in a single refueling operation that lasts for about one minute, the mean air concentration of benzene to which a petrol pump worker is exposed is 3709 $\mu\text{g}/\text{m}^3$. In addition, most of the benzene (88%) is emitted while supplying fuel to the vehicle.¹⁵ Since benzene reactive metabolites can experience redox recycling, they increase the intracellular production of reactive oxygen species (ROS), which would damage cellular macromolecules such as DNA, fats and proteins that result in damaged cell function.¹⁶

Exposures to petroleum products both in and outside petroleum industries have been reported to have some effects on the users, with those who are occupationally exposed being more likely to be affected than their counterparts. The effect of exposure to petroleum fumes on officers' plasma antioxidant defence systems that fill gasoline, there is an increase in lipid peroxidation. Lipid peroxidation, the oxidative catabolism of polyunsaturated fatty acids, is widely accepted as a general mechanism for cellular injury and death, has been

implicated in diverse pathological conditions.¹⁷

The DEPs, the major contributors to air pollution, induce inflammatory responses in the nasal epithelium. Overproduction of airway mucins is an important pathogenic finding in inflammatory airway diseases. The DEPs stimulated the expression of mucin gene MUC4 via the p38/CREB pathway in human lung mucoepidermoid carcinoma cell line (NCI-H292) cells and primary nasal epithelial cells (PNECs). The results of the present study pave the way for further studies on the role of MUC4 in DEP-induced hypersecretion in airway epithelium via the p38/CREB pathway in NCI-H292 cells. The DEPs enhance MUC4 expression in NCI-H292 cells and nasal epithelial cells via the p38/cAMP response element-binding protein (CREB) pathway.¹⁸

Several studies indicated that occupational exposure to specific factors, including exposure to benzene, pesticides and mineral fibers is associated with the risk of cancer development. There is increasing evidence to indicate that the occupational exposure to benzene, pesticides and mineral fibers that may be associated with an increased risk of cancer. Benzene is an aromatic hydrocarbon which is widely used in the production of several polymers, resins and synthetic fibers. It is also a component of wood, gasoline and tobacco smoke. The general population is exposed to benzene through the inhalation of vapors released by motor vehicles, service stations and cigarette smoke. Additionally, contamination can occur due to the ingestion of polluted foods or water. epidemiological and experimental studies have suggested that the exposure to benzene can cause a great number of acute and chronic diseases, which can involve several human tissues or organs. The acute and chronic effects of exposure to benzene can involve the central nervous system,

the reproductive and developmental system, the immune system and the respiratory system. It is widely accepted that benzene can cause hematological diseases, such as acute myeloid leukemia, acute and chronic lymphocytic leukemia, nonHodgkin's lymphoma, multiple myeloma and aplastic anemia. There is evidence to suggest that exposure to benzene is associated with epigenetic alterations investigation of mitotically and meiotically heritable alterations in gene expression without alterations in the DNA sequence.¹⁹

Some international agencies have determined the value of the size of toxicity to xylene. The American Conference of Governmental Industrial Hygienists (ACGIH) determines the value of 100 ppm for 8 h for the time-weighted average (TWA) limit and 150 ppm for 15 min. Not much different, NIOSH (National Institute for Occupational Health and Safety) and Occupational Safety and Health Administration (OSHA) set the same number for TWA that is 100 ppm or about 435mg/m³ and 150 ppm or about 655mg/m³. The molecular weight of ethylbenzene is 106.16 with a boiling point of 136.30. According to the OSHA the exposure time limit for ethylbenzene is 8 hours a day, whereas according to NIOSH limits recommended 10 h a day and 40 h total in 1 week and 125 ppm (545mg/m³) if exposed for a short time. According to ACGIH the threshold exposure to ethylbenzene is 100 ppm (434mg/m³) with time limit of exposure 8 hours a day and 40 hours in 1 week and short exposure limits at 125 ppm (543 mg/m³).²⁰

The intricate mechanism of gasoline-induced adverse effects, including the formation of reactive metabolites via bio-activation and subsequent generation of reactive oxygen species (ROS) and oxidative stress, which are involved in multiple mechanisms that are central to the aetiology of gasoline-induced toxicity. These mechanisms

include covalent binding to DNA, leading to oxidative damage, tumor-suppression gene activity, and activation of pro-oncogenes. Furthermore, it results in induction of autoimmunity and local inflammatory responses, disruption of multiple neurotransmitters and immune cell function, derangement of various enzyme activities (e.g., sodium-potassium adenosine triphosphate (Na⁺/K⁺/ATPase) activity, cytochrome P450 (CYP450), nitric oxide synthase (NOS), antioxidant enzyme activities, etc.), conjugation of bile, and non-specific cell membrane interaction, leading to damage of the membrane lipid bilayer and proteins. Gasoline is a complex mixture of hydrocarbons and additives, including short-chain organic compounds, light-chain volatile compounds, and heavy-chain hydrocarbons however, the relative concentration of gasoline components is dependent upon the crude oil source, refinery process, and production lines used. Toxicological studies indicate that the light-chain volatile compounds (BTEX) are the components most toxic to humans. They constitute the volatile fractions of gasoline that are gradually released into the air and may exist in both the vapor phase and the water-soluble fraction, due to their high vapor pressure and water solubility.²¹

Ethylbenzene may be exposed through inhalation, ingestion, eye or contact direct, and absorption through the skin. If the ethylbenzene vapor level reaches 1000 ppm it causes irritation of the eyes, when 2000 ppm it causes heavy irritation to the eye and irritation of the nasal mucosa. If exposed for a long time at 100 ppm levels it can cause fatigue, headache, slight irritation of the eyes, difficulty in respiration, and dermatitis.²⁰

Benzene, toluene, xylene and styrene (collectively referred to as BTXS) are usually generated during dismantling and incinerating process of electronic wastes. The release of these compounds into ambient air can cause harmful

effects on health and environment. Benzene is considered to be teratogenic, carcinogenic and mutagenic. Long-term exposure of styrene can cause a variety of health problems such as headache, fatigue, weakness, depression, and peripheral neuropathy. Although toluene and xylene have been not yet classified as carcinogens, the increase in rectal and colon cancer incidences has been reported among the exposed population. Besides, these volatile organic compounds (VOCs) can react with nitrogen oxides in the presence of sunlight to produce ozone and peroxyacetyl nitrate, thus lower the air quality.²²

Several previous studies reported the exposure to gasoline vapors on workers at gas stations can cause lung disease. One study on histologic changes with an electron microscope in marmot rodents exposed to gasoline vapors for 30, 60 and 90 days in the laboratory, reported that gasoline vapors cause histologic changes, which increased according to duration of exposure. There was infiltration of inflammatory cells in the mucosa and submucosal trachea, loss cilia in the tracheal epithelium, and an increase in the size of the tracheal submucosal gland, while there was damage and desquamation of the tracheal epithelium, and inflammatory cell inflammation as well decreased number of goblet cells. The electron microscope found shrinkage and disorientation of cilia, and decreases in goblet cell count when compared with the control group. It can be concluded that gasoline vapors damage the mucosa and submucosa trachea, which may reflect also disruption of the lower respiratory tract.²³

Inhaling gasoline vapors may possibly result in irritated nasal mucosal membranes and necrosis. Gasoline fuel vapors with irritant properties cause movement of the cilia to be slow, and even can stop the cilia so they cannot clean the airways. Increased mucus production may also occur due to irritation by

pollutants. This mucus production causes narrowing of the respiratory tract. In addition, the destruction of bacterial killer cells in the airways, the loss of cilia and the swollen lining of the mucous membrane cells will cause difficulties in breathing so that foreign objects including other microorganisms cannot be excreted out of the respiratory tract and this facilitates the occurrence of a respiratory tract infection. This exposure can stimulate inflammatory cells (neutrophils, polymorphonuclear and eosinophil).²⁰

CONCLUSION

This study concluded that the common nasal mucosal cytology features in general refueling station workers can be inflammation, metaplasia and dysplasia. There are significantly differences of metaplasia and dysplasia in nasal mucosa cytology features between gas station workers compared to non-exposed workers, while inflammatory epithelial changes were not significantly different.

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