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Research paper

Application of Bethesda terminology to categorize buccal epithelial smears among petroleum station workers in Taif city, KSA

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Abstract

Background and Objectives: Exposure to Petroleum products has a well-established mutagenicity and carcinogenicity of some components such as benzene. Buccal mucosal cells are capable of metabolizing the carcinogenic compounds to reactive chemicals. We investigated the buccal epithelial cell cytological changes resulting from the occupational exposure to Petroleum derivatives such as benzene.
Methods: Samples were obtained from 70 Petrol station workers in Taif city-KSA, examined using the Papanicolaou stained smears and categorized based on Bethesda Terminology System. Statistical analysis was carried out with the SPSS.
Results: About 38.6% of petrol station workers exhibited buccal cytological changes ranging from atypical squamous cells of undetermined significance (ASC-US) to high-grade squamous intraepithelial lesions (HSIL). Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) was the most frequent cytological abnormality. Buccal smears with abnormal cytological findings dominated among smokers in contrast to non-smokers with a statistically significant difference (P = 0.026). No significant associations were detected between the category of buccal cytological changes and the duration of work or the participant's age.
Conclusion: Bethesda Terminology System can be efficiently applied to buccal smears. Petrol products induce precancerous epithelial changes in buccal epithelial cells indicating a potential health risk for Petrol station workers, thus annual checkup for those workers should be set as primary prevention of occupational-related cancer. Cigarette smoke is a co-factor that exacerbates the effects of Petrol derivatives.

Keywords: *Benzene; Bethesda System Terminology; Buccal Mucosa; Carcinogenic; Smear*.

1. Introduction

Petroleum products are a complex mixture of aliphatic and aromatic hydrocarbons with high volatility. Such types of hydrocarbons exhibit co-mutagenic and co-carcinogenic properties [1]. Exposure to Petroleum (gasoline) vapors is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans, mainly on the basis of the well-established mutagenicity and carcinogenicity of some components such as benzene [2].
Benzene is a widespread environmental chemical with a carcinogenic nature. The current permissible exposure level is 1 part per million (ppm) in air for 8 hours [3]. The association between exposure to benzene or benzene-containing mixtures with acute non-lymphocytic (myeloid) leukemia, hemato-toxicity and other bone marrow disorders has been shown in epidemiological studies in different countries [4], [5]. The carcinogenic mechanisms of benzene have been demonstrated through in vitro models. Some of the reactive metabolites of benzene, such as phenol, catechol, and hydroquinone, can bind to and damage macromolecules, including DNA [6]. These reactive metabolites may also generate reactive oxygen species (ROS) that can exacerbate the DNA damage [7].
Additionally, alterations in DNA methylation -notably mitochondrial DNA- have been recently verified to result from low-dose benzene exposure as a direct effect of ROS-induced DNA damage [8].
Exposure to benzene in human populations can occur in various work-related settings in which benzene is used or produced, or from traffic emissions resulting from incomplete combustion of fossil fuel, or from other sources. Work-related exposures to benzene occur among Petrochemical laboratory workers and Petrol station workers who pump fuel to vehicles and absorb the products of fuel fumes and the products of combustion [9, 10]. Cigarette smoke is an important source of benzene in indoor air. It represents one of the factors that may influence the rate of benzene exposure-induced cytogenetic damage [10].
Buccal mucosal cells are capable of metabolizing the carcinogenic compounds to reactive chemicals [10]. Additionally, recent studies have demonstrated that petroleum derivatives are able to induce genetic damage and cellular death including micronuclei; binucleation and cellular death (pyknosis, karyolysis and karyorrhexis) in buccal mucosal epithelial cells from gas petrol attendants [10], [11].
The Bethesda System Terminology is a system applied for reporting cervical or vaginal cytological preparations. It was introduced

in 1988 and revised in 1991 and 2001. The Bethesda System Terminology adopted in 2001, includes revisions in statements of adequacy, general categorization, and interpretation and results of epithelial cell abnormalities. The system categories include: unsatisfactory for evaluation (inadequate), negative (normal), atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL) [12].

The buccal epithelium is composed of four strata including the basal cell, prickle cell, intermediate and superficial layers that are continually renewed by proliferation and migration of the basal cells to the surface [13]. Owing to the close histological similarity of buccal epithelium to cervico-vaginal epithelium and the lack of a standardized method for reporting oral cytology, many investigators used an oral cytological grading system on adequate samples in a similar classification to the Bethesda System Terminology [14].

2. Objectives

To investigate the buccal epithelial cell cytological changes resulting from the occupational exposure to Petroleum derivatives such as benzene. Samples were obtained from a population of 70 Petrol station workers in Taif city-KSA, examined using the Papanicolaou stained smears and categorized based on the categories of Bethesda Terminology System. Frequencies of different terminology categories were compared in both smokers and non-smokers as well as to the age of the participants and the duration of exposure in order to examine the possible effects of these co-factors.

3. Materials and methods

3.1. Study population

The study population comprised a total of 70 healthy males (10 smokers and 60 non-smokers) working at petrol stations located in Taif city-KSA. Participants were informed in detail about the study plan and written informed consents were obtained. All subjects were selected based on standardized questionnaire which included questions about age, working duration associated with occupational exposure, smoking habit, using drugs, alcohol, medical history, and radiological exams. Subjects with oral lesions were excluded from the study.

3.2. Buccal cell sampling, preparation and staining

Buccal cell samples were collected at the end of the work shift (8 hours exposure). Participants were asked to rinse their mouth with water before sampling to remove unwanted debris. Wooden spatulas were used to obtain cell samples from buccal mucosa [10]. The samples were then applied to clean microscope slides. Each slide was labelled with number of the participant according the order in the questionnaire. Samples were smeared rapidly and gently on the glass slides to avoid drying the cells, which may cause marked artifacts that can preclude an adequate cytological evaluation. Smears were rapidly immersed in 95% ethanol for 15 minutes which is the method of choice [15]. In the laboratory, the Papanicolaou stained smears were prepared as described by Bancroft [16].

3.3. Assessment and categorization of cytological smears

Cytological results were classified as unsatisfactory for evaluation (inadequate), negative (normal), atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells but cannot exclude high-grade squamous intraepithelial lesion (ASC-H), low-grade squamous intraepithelial lesion (LSIL), and high-grade squamous intraepithelial lesion (HSIL). Evaluation criteria for buccal cytology used in this study followed 2001 Bethesda System Terminology [15], [17].

3.4. Statistical analysis

Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS) version 16.0 (Chicago, USA). The associations between the buccal cytological findings with smoking habit, age and duration of working were assessed by the Pearson Chi-square test $\chi 2$ test. A statistically significant difference was accepted at a p-value of ≤ 0.05.

4. Results

The study included 70 Petrol station male workers, ranging in age between 19 and 55 years with a mean age of 30.26 years. About 14.3% of the study group were smokers (Table1). The participants were classified into age groups (Table 2). The duration of working among the study group ranged from less than one year, to above 10 years. Almost half of study group (50%) worked for duration from one to five years (Table 3).

According to Bethesda system terminology, the buccal smears were categorized into inadequate (unsatisfactory; 7.1%), negative (normal; 54.3%), atypical squamous cells of undetermined significance (ASC-US; 4.3%), atypical squamous cells but cannot exclude high-grade squamous intraepithelial lesion (ASC-H; 22.9%), low-grade squamous intraepithelial lesion (LSIL; 7.1%), and high-grade squamous intraepithelial lesion (HSIL; 4.3%) (Fig.1-5). ASC-H was the most frequent cytological abnormality.

As seen in table 1, buccal smears with abnormal cytological findings dominated among smokers in contrast to non-smokers with a statistically significant difference between smokers and non-smokers ($P = 0.026$).

Table 1: Frequency of Buccal Smear Cytological Changes among 70 Petrol Station Workers in Relation to Smoking Habit

Smoking habit	Bethesda System Terminology						
	Inadequate	Normal	ASC-US	ASC-H	LSIL	HSIL	Total
Smoker	1 (10.0%)	1 (10.0%)	1 (10.0%)	5 (50.0%)	1 (10.0%)	1 (10.0%)	10 (100.0%)
Non-smoker	4 (6.7%)	37 (61.7%)	2 (3.3%)	11 (18.3%)	4 (6.7%)	2 (3.3%)	60 (100.0%)
Total	5 (7.1%)	38 (54.3%)	3 (4.3%)	16 (22.9%)	5 (7.1%)	3 (4.3%)	70 (100.0%)

Table 2: Frequency of Buccal Smear Cytological Changes among 70 Petrol Station Workers in Relation to Age

Age groups	Bethesda System Terminology						
	Inadequate	Normal	ASC-US	ASC-H	LSIL	HSIL	Total
19-29y	4 (9.5%)	21 (50.0%)	2 (4.8%)	12 (28.6%)	1 (2.4%)	2 (4.8%)	42 (100.0%)
30-39y	0 (0.0%)	9 (50.0%)	1 (5.6%)	4 (22.2%)	3 (16.7%)	1 (5.6%)	18 (100.0%)
40-55y	1 (10.0%)	8 (80.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)	0 (0.0%)	10 (100.0%)
Total	5 (7.1%)	38 (54.3%)	3 (4.3%)	16 (22.9%)	5 (7.1%)	3 (4.3%)	70 (100.0%)

With regard to the association between age groups and cytological findings, most of abnormal findings (Table 2) were found among the age group between 19 to 29 years followed by the age group between 30 to 40 years however the association was not statistically significant ($P > 0.05$).

Table 3 shows the distribution of the cytological findings in relation to the duration of working. It was noticed that low and high

grad squamous intraepithelial lesions (LSIL&HSIL) were reported mainly among the participants who worked in petrol stations from one to five years; however this observation was statistically insignificant (P > 0.05).

Table 3: Frequency of Buccal Smear Cytological Changes among 70 Petrol Station Workers in Relation to the Working Duration

Duration of working	Bethesda System Terminology						
	Inadequate	Normal	ASC-US	ASC-H	LSIL	HSIL	Total
Below 1y	2 (13.3%)	7 (46.7%)	1 (6.7%)	4 (26.7%)	1 (6.7%)	0 (0.0%)	15 (100.0%)
1-5y	2 (5.7%)	22 (62.9%)	1 (2.9%)	7 (20.0%)	2 (5.7%)	1 (2.9%)	35 (100.0%)
6-10y	1 (9.1%)	7 (63.6%)	0 (0.0%)	1 (9.1%)	1 (9.1%)	1 (9.1%)	11 (100.0%)
Above 10y	0 (0.0%)	2 (22.2%)	1 (11.1%)	4 (44.4%)	1 (11.1%)	1 (11.1%)	9 (100.0%)
Total	5 (7.1%)	38 (54.3%)	3 (4.3%)	16 (22.9%)	5 (7.1%)	3 (4.3%)	70 (100.0%)

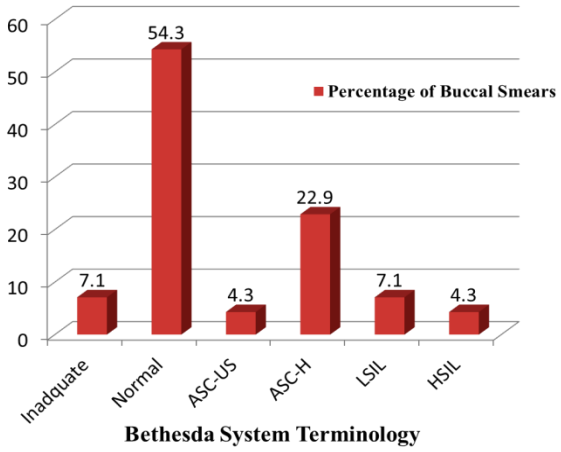

Fig. 1: Frequency of Buccal Smear Cytological Changes among 70 Petrol Station Workers according to the Categories of Bethesda System Terminology.

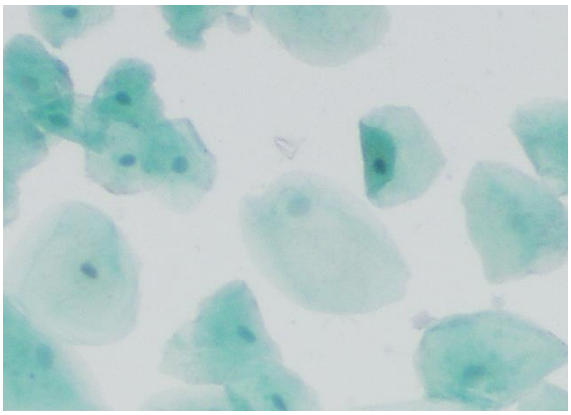

Fig. 2: Normal Buccal Pap smear (Papanicolaou, × 400).

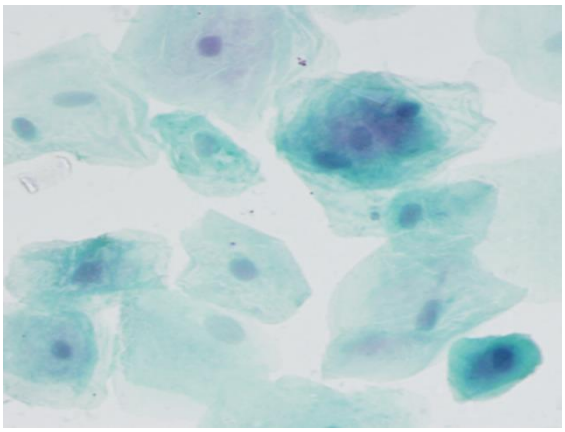

Fig. 3: Buccal Pap smear with Atypical Squamous Cells of Undetermined Significance (ASCUS) Showing Nuclear Enlargement One &Half to Two Times (Papanicolaou, × 400).

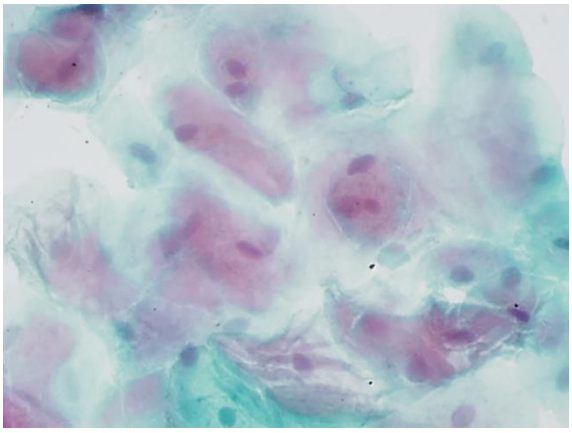

Fig. 4: Buccal Pap smear with Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H), Showing Nuclear Enlargement, with Possible Variation in Nuclear Size and Shape and Binucleation (Papanicolaou, × 400).

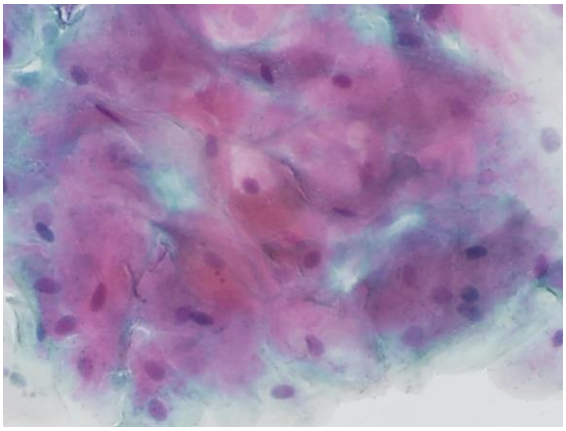

Fig. 5: Buccal Pap smear Showing High-Grade Squamous Intraepithelial Lesion (HSIL). The Cells are Immature, Crowded, Forming Sheets/Groups (Papanicolaou, × 400).

5. Discussion

Petrol station worker is a representative of an occupational group who permanently exposed to hazardous air pollutants (HAPs). This worker is exposed to various gasoline products via different routes, e.g. inhalation; ingestion and dermal absorption [18]. The hazardous effect of benzene exposure includes genotoxicity, neurotoxicity, hematotoxicity and carcinogenesis [19].

The oral mucosal permeability in different regions of the mouth is an important aspect to consider when analyzing the local effects of carcinogenic agents. The non-keratinized tissues, such as the buc-

cal mucosa are much more permeable than keratinized tissues, such as the palate and gingiva [10].

Compared to cytogenetic techniques, cytological analysis of exfoliated buccal mucosal cells using Papanicolaou smears provides a non-invasive, cost- limited, and time- saving tool for evaluating cytological changes. Moreover, this method is relatively easy in interpretation.

When concerned about cervical cancer screening, Pap test is widely accepted as an effective screening tool for cervical precancerous lesions and cancer [15]. However, there is still no definite grading recommendation offered for oral cytological changes which may progress to cancer. This may be attributed to lack of interest in oral cytology or the high rate of false results caused by inadequate sampling procedures, low cellularity or variation in technical quality [14]. In settlement with our approach, a previous study adapted Papanicolaou smears to categorize anal intraepithelial lesions using Bethesda System Terminology [15].

In this study, we have demonstrated that about 38.6% of petrol station workers exhibit buccal cytological changes ranging from ASC-US to HSIL, which are approved to be precancerous changes in accordance with the Bethesda System Terminology applied for cervical epithelial lesions. These abnormal cytological findings were more remarkable among smokers in contrast to non-smokers with a statistically significant difference. This finding gives support to Celik et al. [20] and Fustinoni et al. [21] who approved that cigarette smoking gives the prevailing contribution to individual genotoxic burden. Additionally, Sudha et al. [22] concluded that cigarette smoke significantly enhanced the genotoxicity and DNA damage of buccal epithelial cells among metal arc welders. On the other hand Hallare et al., [23] and Tunsaringkarn et al. [18] showed no correlation between different genotoxic effects of benzene exposure and the smoking habit, duration of exposure and age. In accordance with the previous two studies, we were unable to detect a significant association between the category of buccal cytological changes and the duration of work or the participant's age.

6. Conclusion

Our findings indicate that Bethesda Terminology System can be efficiently applied to buccal smears. Exposure Petrol products specially benzene, induce epithelial changes in buccal epithelial cells, indicating a potential health risk for Petrol station workers, thus annual check-up and monitoring for benzene exposure among the Petrol station workers should be set as primary prevention of occupational-related cancer for them. Furthermore, it is important to consider the effects of other factors that exacerbate the effects of Petrol derivatives such as cigarette smoke.

7. Disclosure

No relevant financial affiliations or conflicts of interest to disclose.

8. Compliance with ethical standards

All procedures performed in study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

9. Informed consent

Informed written consent was obtained from all individual participants included in the study.

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