Cytotoxicity in Exfoliated Buccal Cells of Petrol Stations' Workers in Erbil City

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***Abstract*—*Petrol* station's workers who pump fuel to vehicles expose to the products of fuel fumes and the products of combustion. Petroleum derivative consists of a complex mixture of chemical compounds. To study the effects of occupational exposure to petroleum derivatives such as benzene, exfoliated buccal cells from 28 petrol station attendants and 30 control subjects were taken and examined for micronucleus (MN) cells frequency. Frequencies of cell's nuclear abnormalities (NAs) other than micronuclei, such as binucleated (BN) cells and karyolysis (KL), were also evaluated. For each, 100 exfoliated buccal cells were analyzed. Analysis of buccal cells revealed that MN and NA frequencies in petrol station workers were significantly higher than in control subjects ($P < 0.001$). This study demonstrates that, using MN assay, it is possible to assess the cytogenetic damage in exposed individuals and that the significant increase in the induction of the MN in the exposed population suggests that the studied individuals may be at a higher risk of cytogenetic damage and therefore monitored for any long-term adverse effects of the exposure.**

***Index Terms*–Buccal cells, Micronucleus test, Petrol station workers.**

I. Introduction

Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments or intact whole chromosomes lagging behind in the anaphase stage of cell division. Their presence in cells is a reflection of structural and/or numerical chromosomal aberrations arising during mitosis [1-3].

Micronuclei and other nuclear abnormalities (NAs) are biomarkers of genotoxic events and chromosomal instability and are collectively measured in micronucleus (MN) cytome assay. The molecular mechanisms behind these events have been investigated using molecular probes and genetically engineered cells. The presence of these nuclear anomalies could increase the risk of developmental and degenerative diseases [4]. There are many bioassays that used to evaluate the impact of environmental factors, genetic, and lifestyle on genomic stability in humans, among these bioassays the MN assay has detached by its ease of use and a low cost associated the precision of viewing a larger number of cells [5].

Benzene, an important component of petrol, is a widely distributed environmental contaminant. Today, about 98% of the benzene is derived from the petrochemical and petroleum refining industries. Therefore, occupational exposure to benzene in humans generally takes place in factories, refineries, and other industrial settings. Moreover, the general population is exposed to benzene contained in petrol, vehicle exhaust, diesel fuel, and cigarette smoke [6].

Exposure to gasoline vapors is classified by the International Agency for Research on Cancer as possibly carcinogenic to humans, mainly on the basis of the well-established carcinogenicity of some components such as benzene [7]. The association between exposure to benzene or benzene-containing mixtures and certain types of leukemia has been shown in epidemiological studies in different countries [8,9].

The buccal epithelium is composed of four strata including the basal cell layer, prickle cell layer, and intermediate and superficial layers. The oral epithelium maintains itself by a system of continuous cell renewal in which new cells produced by mitosis in the basal layer migrate to the surface to replace those that are shed. Thus, the mucosa is composed of progenitor and maturing cell populations [10].

Petrol station workers are chronically exposed to petroleum derivatives primarily through inhalation of the volatile fraction of petrol during vehicle refueling. Taking into account that occupational exposure to such derivatives may possess genotoxic risk, the aim of our study is to investigate the cytogenotoxic damage in exfoliated buccal cells from petrol station workers and control subjects, using the MN test in Erbil city, Kurdistan region, North of Iraq.

II. Materials and Methods

A. Subjects

This study was carried out on 28 petrol station workers at 7 petrol stations (Akar, Woza, Salam, Bakor, Nissan, Sher, and

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Jabbar) located in the center of Erbil city. The control group consisted of 30 healthy students of Biology Department, College of Science, University of Salahaddin-Erbil, without indication of any exposure to petroleum derivatives or other potential genotoxic substances. Participants were informed about the study and asked to complete a standardized questionnaire to obtain necessary data on lifestyles and personal factors (age, working period, health, etc.).

B. Evaluation of Micronuclei and NAs in Buccal Cells

Buccal cell samples were collected during working time at petrol stations. Subjects were asked to rinse their mouth with water before sampling. A clean cotton plastic spatula was used to obtain cell samples from buccal mucosa. The samples were then applied to clean microscope slides. Smears were air dried and fixed in methanol for 5 min. Slides were stained by the Giemsa stain reaction technique according to Stich and Rosin, 1984 [11]. Two slides were prepared for each subject and 100 cells were evaluated per slide to determine the MN frequencies.

NAs were classified according to Tolbert, *et al*., 1992 [12]. Briefly, cells with two nuclei were considered as binucleates (BN). Nuclear dissolution, in which a Giemsa-negative, ghost-like image of the nucleus remains, was evaluated as karyolysis (KL). The following criteria for MN analyses were used in oral epithelial cells. An MN must (i) be less than one-third the diameter of the main nucleus; (ii) be on the same plane of focus; (iii) have the same color, texture, and refraction as the main nucleus; (iv) have a smooth, oval, or round shape; and (v) be clearly separated from the main nucleus.

C. Statistical Analysis

All the data are expressed as the mean ± standard error of the mean. Data were tested for normality by the Kolmogorov–Smirnov and Shapiro–Wilk test. The data were analyzed statistically using the Student's *t*-tests for independent samples. $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the program SPSS 18 for the PC.

III. RESULTS AND DISCUSSION

Results for micronuclei and NAs are shown in Table I and Figs. 1-5. Assessment of MN frequencies in exfoliated buccal cells revealed a significant difference ($P < 0.001$) between exposed workers (19.25 ± 0.89) and control subjects (2.40 ± 0.35). A significant difference in binucleus frequency was observed between controls (0.40 ± 0.09) and exposed (1.46 ± 0.33) workers ($P < 0.001$). KL frequency was found to be significantly higher ($P < 0.001$) in exposed individuals (12.71 ± 0.73) than controls (0.70 ± 0.12).

However, the data on cytogenetic damage in petrol station attendants are rather controversial. Evaluation of sister chromatid exchange (SCE) frequencies in petrol station attendants showed that there was no significant difference between controls and experimental subjects [13,14]. Bukvic, *et al*., 1998 [15], also analyzed MN and SCE frequencies

TABLE I COMPARISON OF MICRONUCLEI AND OTHER NUCLEAR ABNORMALITIES IN EXFOLIATED BUCCAL CELLS OF CONTROLS AND EXPOSED SUBJECTS (MEAN±STANDARD ERROR)

Parameters	Controls	Workers	*P*-value
MN	2.40±0.35	19.25±0.89	0.001
BN	0.40±0.09	1.46±0.33	0.001
KL	0.70±0.12	12.71±0.73	0.001

MN: Micronucleus, BN: Binucleated, KL: Karyolysis

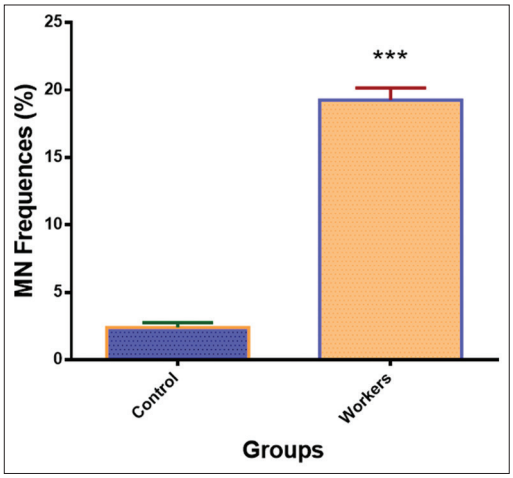

Fig. 1 Frequency of distribution of micronuclei in petrol station workers and control subjects.

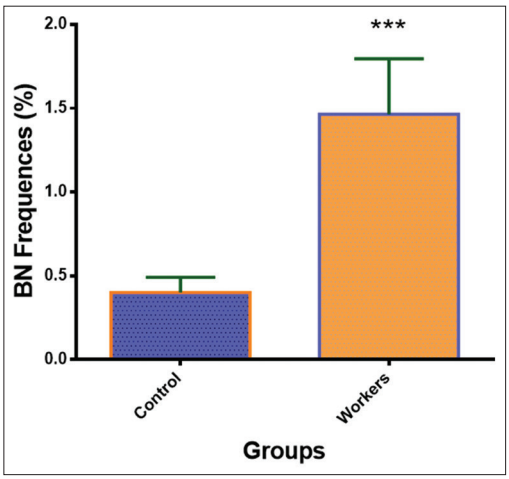

Fig. 2 Frequency of distribution of binucleates in petrol station workers and control subjects.

in peripheral lymphocytes of petrol station attendants and failed to reveal significant differences for SCE, although they found significant differences for MN. Nevertheless, in another study, no significant increase in MN frequency in cultured blood lymphocytes from a group of filling station attendants was detected [16]. Surralles, *et al*., 1997 [17], performed a molecular cytogenetic analysis on buccal cells and lymphocytes obtained from benzene-exposed workers employed in a petrochemical complex in Estonia and reported that there was no increase in the frequency of cytogenetic damage with respect to the controls.

In the current study, significant increases were observed in the frequency of micronucleated buccal cells in petrol station

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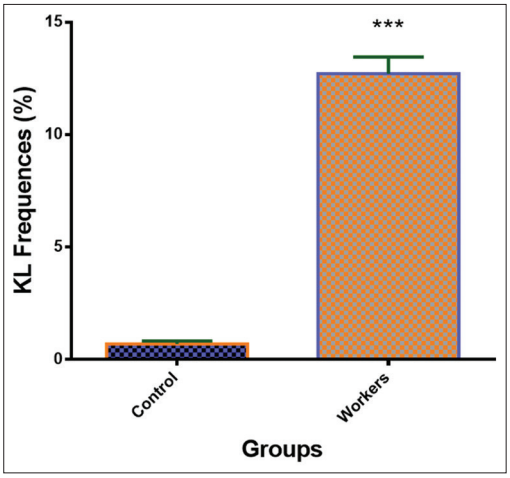

Fig. 3 Frequency of distribution of karyolysis in petrol station workers and control subjects.

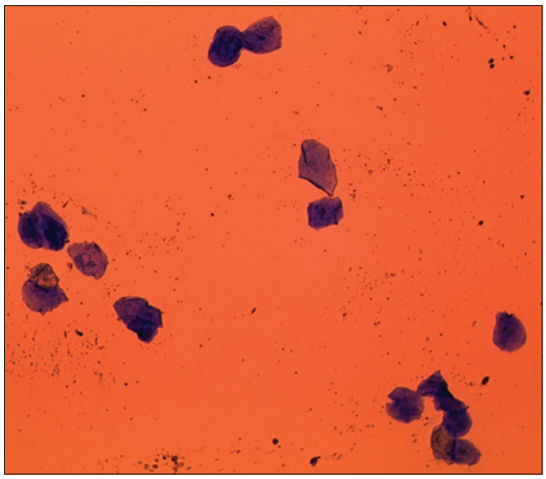

Fig. 4 Oral mucosa cells (ocular 10x, objective 10x).

workers. These findings are in agreement with those of Celik, *et al.*, 2003 [18], who detected a significant increase in the frequency of micronuclei and NAs in the buccal cells of petrol station workers. Furthermore, our findings are in agreement with those of Metgud, *et al*., 2015 [19], and Kurteshi, *et al*., 2017 [20], whom detected a significant increase in the frequency of micronuclei in the exfoliated buccal cells of petrol station workers. Similarly, Arul, *et al*., 2017 [21], also found that micronuclei in exfoliated buccal cells of petrol station workers increased, using liquid-based cytology method.

Analysis of exfoliated cells of buccal mucosa also provides evidence of other NAs such as BN (presence of two nuclei within a cell) and KL (nuclear dissolution) [12]. Binucleus formation is considered as indicator of cytotoxicity, while KL is considered as indicator of apoptosis. In our study, a comparison of NAs also revealed significant differences between controls and exposed subjects. Similar results were observed for buccal cells of fire breathers exposed to diesel [22].

Benzene exposure of petrol station attendants can vary widely due to several factors such as quantity of fuel pumped, type and number of vehicles filled, protective measures, and

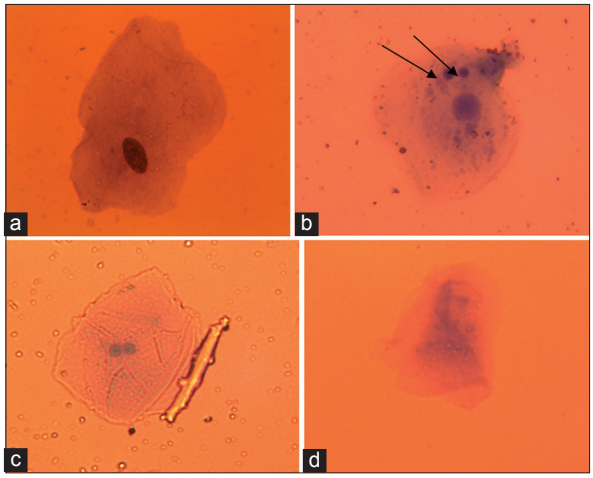

Fig. 5 (a) Cells with normal nucleus. (b) Micronucleated cell (MN), (c) Binucleated cells (BN), and (d) Karyolysis (KL). Photomicrographs stained with Giemsa viewed at 1000 (ocular:10x, objective:100x). magnification under compound binocular microscope, Olympus.

total content of benzene in the petroleum. On the other hand, it should also be emphasized that petrol station attendants are not only exposed to hydrocarbons present in petrol vapors but also to the emissions produced by engines during fuel combustion. It was shown that these emissions may also cause cytotoxic and genotoxic effects [23].

In conclusion, results make it clear that the petrol station workers had a significant increase in cytogenetic damage as tested by MN assay. The MN assay in human exfoliated buccal cells is one of the effective methods used for detecting cytogenetic abnormalities in human populations. As demonstrated in this study, other NAs, such as BN and KL, are also useful indices of chemical exposure and toxic response. Therefore, a combination of micronuclei and NAs may increase the sensitivity of the exfoliated epithelial cell technique in the assessment of genotoxicity.

A higher frequency of buccal cells with micronuclei, BN, and KL was observed in the study subjects, probably due to the genotoxic effect of the petroleum derivatives to which they are exposed. It is necessary to educate the working population about the genotoxic effects of petrol exposure and to ensure safe and healthy working atmosphere for the petrol station workers to alleviate the health hazards that they may encounter.

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