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Research Article

Micronucleus Frequency in Buccal Cells of Males Exposed to Air Pollution in Kufa City

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Abstract

The aim of the present study is to explore micronuclei recurrence, as a biomarker of genomic damage in buccal cells of individuals living in polluted areas located near to the cement factory in Kufa city. Twenty four apparently healthy males residing in Kufa are enrolled in the present study, their ages between 18-30 years, as control group 24 males with same age are enrolled living in the center of Najaf city a far from cement factory. A sample of the exposed and control groups of buccal mucosa cells was collected during Spring months in 2016. The mean count of cell with micronuclei in buccal cells of the exposed group and control were (22.33 ± 0.97; 11.583 ± 0.85) respectively, the mean count of the binucleated cells of the exposed and control groups were (7.208 ± 0.71, 10.041 ± 0.84) respectively, a significant difference was detected in the observed frequencies of micronuclei and binnucleated cells in the buccal mucosa cells between the exposed and control group. The results indicated that exposure to air pollutants related to cement production induce cytotoxic as well as genotoxic damage in buccal mucosa cells.

Keywords: Kufa, micronuclei, binucleated cells, air pollution.

الخلاصة

الهدف من الدراسة الحالية هو التحري عن تكرار النوى الصغيرة في الخلايا الطلائية المبطنة للفم كمؤشر لمقدار الضرر في المادة الوراثية لمجموعة من سكان منطقة ملوثة بالقرب من معمل أسمنت الكوفة, شملت الدراسة اربعة وعشرون ذكرا من سكان منطقة الكوفة ,كانت اعمارهم تتراوح بين 18-30 سنة, كما شملت الدراسة ايضا اربعة وعشرون ذكرا من سكان مدينة النجف وبنفس الفئة العمرية وعدت كمجموعة سيطرة. تم جمع العينات من الافراد المتعرضين والسيطرة خلال اشهر ربيع 2016. كان معدل الخلايا ذات النوى الصغيرة في الخلايا المبطنة للفم للأفراد المعرضين والسيطرة (22.33± 0.97 85;) 11.583 ± 0.) ومعدل الخلايا ثنائية النواة للأفراد المعرضين والسيطرة (10.041 ± 0.84; 7.208 ± 0.71) لوحظ وجود اختلافا معنويا بتكرار ظهور النوى الصغيرة والخلايا ثنائية النواة في الخلايا المبطنة للفم عند مقارنة الافراد المعرضين مع السيطرة. دلت النتائج الى ان التعرض الى ملوثات الهواء المصاحبة لإنتاج الاسمنت يستحث سمية خلوية ووراثية للخلايا المبطنة للفم.

Introduction

Air pollution is a combination of inconsistent concentrations of gases and solid particles in the air. Air pollution and particulate matter are globally recognized as carcinogenic to human [1] [2]. During the previous decades, many literatures have evaluated the genotoxic impacts of air pollution among population residing in areas with medium or high levels of air pollution [3] [4].
Numerous studies have utilized the micronucleus test to determine an association between pollution and cytotoxic and/ or genotoxic effects [5] [6]. In the eighties the buccal cell micronuclei test recommended as a biomarker for genomic damage. Subsequently, this test chosen as convenient biomarker of chromosomal damage created by exposure to different environmental pollutants, lifestyle habits in addition to inherited diseases [7]. In the last decade the micronuclei tests in buccal cell have gotten increasing popularity among laboratories working in the field of environmental mutagenesis consequently, the number of published researches based on this biomarker has expanded. Previous research demonstrates that an elevation in the micronucleus score in buccal cells denotes to an increase in risk for malignancy [8] [9]. In Iraq, there are no studies have considered the genomic damage in mucosa buccal cells as micronuclei(MN) score in cells of the population exposed to air pollution. The aim of the present work is to explore micronuclei recurrence, as a biomarker of ge-

nomic damage in buccal cells of individuals living in polluted areas located near to the cement factory in Kufa city.

Materials and methods

The present study includes twenty four apparently healthy males residing in Kufa near cement factory (Al-Kūfa is a town in the south of Baghdad northeast of Najaf city it is situated on the banks of the Euphrates river). Their ages between 18-30 years, as control group 24 males with same age are enrolled living in the center of Najaf city a far from cement factory. All study participants were informed of the study objectives. The data about date of birth, occupational history, health status Lifestyle (smoking, drinking habits and diet) was provided by questionnaire.

A sample of the exposed and control groups of buccal mucosa cells was collected during spring months in 2016. Before cell collection all volunteered were asked to rinse their mouth thoroughly with water to get rid of any unwanted debris. Smooth toothbrushes were used, the head of the toothbrushes was swapped on clean slides, and then samples were fixed with ethanol (90%) for 48 h, and stained with Giemsa for 20 minutes. To count the cells with micronuclei and binucleate cell per 2000 cells for each individual stained slide scanning by light microscope (at 400 X magnification). This procedure was done according to the criteria originally described by Tolbert *et al.* [10] [11].

Statistical Analysis:

To analyze the data The Statistical Analysis System- SAS [12] program was used. Least significant difference –LSD test was used to compare the means of micronuclei and binucleated cells between the studied groups.

Results and Discussion

A cell with micronuclei was differentiated by the presence of one or more than one a small additional nuclei in the cytoplasm (Figure 1). The main finding of the present work was a surprisingly in an elevation score of micronuclei in buccal cells of male living in exposed area. As shown in Table (1) the mean count of cell with micronuclei in buccal cells of individuals from exposed area was (22.33 ± 0.97). It was significantly higher in comparison with the micronuclei frequency of control subjects ($P<0.0001$).

According to preceding researches that included adults and children, the range of micronuclei score in buccal cell in unexposed subjects interval of between (3-17 /2000 cell) [13]. Hence, the mean count of cell with micronuclei found in the present study is about two- fold higher than reference value for unexposed individuals. Additionally, the mean value of the binucleated cells of males from exposed area was (10.041 ± 0.84) also significantly higher ($P < 0.01$) in comparison with mean value of binucleated cell in the control subjects.

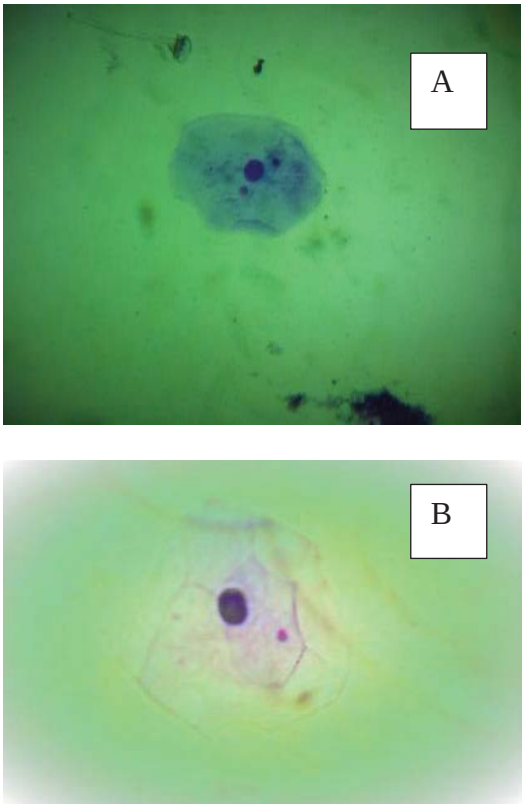

Figure1: A: buccal cell with two micronuclei, B: buccal cell with one micronuclei (400X magnification).

Binucleated cells are cells containing two very close main nuclei instead of one and they are considered marker of cytokinesis failures or cytokinetic faults as a result of aneuploidy [7]. Increased frequency of binucleated cell of the male of exposed area indicated that exposure to air pollutants induce cytotoxic as well as genotoxic damage. Our results are consistent with Jara-Ettinger *et al.* [5] who report a significant increase of binuclated score in Mexican wilder.

Al-Kufa cement factory located near urban centers and may create a serious hazard to the environment as well as human health [14].

The main air pollutants related to cement production include: sulfur oxides, nitrous oxides,

carbon oxides, particulates and organic compounds in addition to cement kilns release large amounts of dust which possibly contains large concentrations of heavy metal and particulates[15] [16]. In fact, most of these compounds have mutagenic and / or cytotoxic effects for that reason it is difficult to conclude which of them represent the effects seen in the present work. The result obtained in the present work are in agreement with previous studies Ceretti *et al.* [17] reported that the rate of micronuclei occurrence in epithelial buccal cells significantly associated with concentration of air pollutants, for example, particulate matter (PM10, PM2.5) and nitrous oxide NO_2. Furthermore an increase of micronuclei score has been detected in peripheral blood lymphocytes as well as in buccal mucosa cells of individuals exposed to urban air pollution[3] [4] [6] [18].

As mentioned above, SO_2 is one of the air pollutants related to cement production; previous study documented an increase of chromosomal aberration in lymphocytes of subjects who were occupationally exposed to SO_2. Numerous studies were published which revealed that SO_2 induces DNA instability in laboratory animals as well as in cultured human cells and it was suggested that SO_2 exposure was responsible to increased risk of lung cancer in workers [19] [21] [22].

Furthermore, the SO_2 also induces genomic instability in plant cells [23]. The impact of cement industry emission on plant production and soil properties has been studied by some researchers, Salama *et al.* [15] documented that cement industry emission includes NO_2, O3 and SO_2 intensely impact the morphology and physiology of *Datura innoxia* plant., Al-Omran *et al*., [24] reported an increase of the Cr, Cd, Ni, and Pb in the soil surrounding the cement plant in Saudi Arabia.

The results of present study showed elevation score of micronuclei and binucleated cells in males living near cement factory. It is in consonance with the finding of earlier studies where researchers utilized the micronuclei test in oral cells to detect the genotoxic impact of air pollution in outdoor workers including tunnel worker, street vendors, driver and gas station attendants [18].

In conclusion the results reveal that first, this micronuclei assay can be utilized in monitoring studies second, supports the need to additional researches shed light on the genotoxic and cytotoxic impact of air pollution on population residing in areas with medium or high level of air pollution.

Table (1): Micronucleated and Binucleated cells frequencies in exposed and control males

Parameter /2000 cell	Exposed males 24 (mean ± S. E)	Controls (unexposed males) 24 (mean ± S. E)
Micronucleated cells	22.33 ± 0.97***	11.583 ± 0.85
Binucleated cells	10.041 ± 0.84*	7.208 ± 0.71

Conclusion

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