ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Monitoring genotoxicity among traffic policemen in bagdhad using the micronucleus assay in exfoliated buccal cells

Israa Hussein Hamzah; Rawaa Abdul-Ameer Abdul-Jabbar; Farha A. Ali Shafi
Department of Biology, College of Science, Mustansiriyah University/Iraq

Abstract:

The present study has been designed to evaluate cytogenetic damage in traffic policemen in Baghdad city, Iraq, by using the Buccal Micronucleus Cytome (BMCyt) assay. Buccal cells have been collected from 51 male participant working across different streets in Baghdad, with age range of 30-45 years. The control group has included 22 age and sex- matched sample of indoor office workers. Quite opposite to expectation, our data have shown no statistically significant differences in the number of micronucleated and karyolytic cells between the exposed and the control groups. Moreover, the data have failed to illustrate differences among smokers and non-smokers with respect to frequency of micronuclei and karyolytic cells, both within the exposed and the control groups. This lack of significant differences might be due to the relatively small size of the sampled group. Further studies with higher number of participants and with the exclusion of smokers should be performed in order to detect effects of exposure to high levels of genotoxic and cytotoxic compounds in the air if urban areas. To our knowledge, the present study is the first attempt to use the BMCyt assay to explore the association between exposure to outdoor air pollution and DNA damage in buccal mucosa cells of traffic policemen in Iraq and it will serve as a base for future studies.

Key words: air pollution, micronucleus assay, traffic policemen, Baghdad

INTRODUCTION :

Outdoor air pollution is increasingly recognized as a serious worldwide public health concern and has been reported to cause more than three million premature deaths per year. This number is expected to increase after the year 2050 as a result of the increase in urban populations and number of cars increased(1).

Outdoor air pollution is a combination of various pollutants originating from a numerous of anthropogenic as well as natural sources. Numerous environmental chemical compounds have been identified in the ambient atmosphere, containing several mutagenic and/or carcinogenic agents, such as heterocyclic compounds, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and particulate matter containing several heavy metal ions such as vanadium and lead (2).

Epidemiological studies have found a close link between long term exposure to outdoor air pollution and incidence of cardiovascular disease and lung cancer. In June 2012 The International Agency for Research on Cancer categorized outdoor air pollution and particulate matter as carcinogenic to humans based on abundant evidence of carcinogenicity in experimental animals and humans(1,3).

Some occupational group, including bus drivers, garage workers, tunnel workers , ferrymen and traffic policemen are exposed to a high level of air pollution, since they expend most of their working time outdoors. Therefore, many biomonitoring studies have been performed on these occupational group using different biomarkers including DNA adduct and cytogenetic alteration(4,5,6). Over the past decade most research in epidemiological studies has emphasized the use of the Buccal Micronucleus Cytome (BMCyt) assay to evaluate chromosomal damage of human population exposed to genotoxic and cytotoxic compounds. BMCyt assay has been also performed in many occupational studies since buccal cells were reported to come in direct contact with many genotoxic and cytotoxic compounds via inhalation and gestation. Additionally, the assay offers a simple and fast non-invasive system that allows detecting other nuclear alterations such as nuclear buds (NBs) that result from gene amplification, binuclear cells (BNs) caused by disorders in the mitotic cycle, and other anomalies denoting cytotoxic effects such as karyorrhexis and karyolysis(KL) (7,8,9). Baghdad is located at the course of Tigris river in the mid-east of Iraq with approximately 8 million inhabitants according to 2014 estimation(10). The city is subject to emissions generated by exhausts from huge numbers of vehicles as well as electric generators of different sizes and uses that have been distributed all around the city due to substantial loss of electricity after the war of 2003. Hence the present study aimed to evaluate the cytogenetic damage in traffic policemen in Baghdad city by using the Buccal Micronucleus Cytome (BMCyt) assay .

SUBJECTS, MATERIALS AND METHODS

Buccal cells have been collected from 51 male participant working across different streets in Baghdad, with age range of 30-45 years. The control group has included 22 age and sex- matched sample of indoor office workers. All samples of buccal mucosa cells were collected during winter 2017. All participants were informed about the objectives of the study and signed an informed consent form. Prior to sample collection, information about age, tobacco consumption, and chronic medical therapy were obtained. Prior to cell collection, participants were asked to rinse their mouths with water. Exfoliated buccal mucosa cells were collected from each subject by gentle scraping of inner lining of left and right cheeks using soft toothbrush. Cells were smeared on clean slide and allowed to dry , fixed in ethanol(80%) for forty eight hours and then stained with Giemsa stain. The analyses of micronucleated cell and nuclear anomalies were done under a total magnification of X1000 using light microscope .By following the criteria developed by Tolbert *et al.*[11,12], a total of 1000 non- fragmented staining cells were counted per subject in order to determine the frequency of micronucleatedand karyolytic cells per 1000 cells.

Statistical Analysis:

The collected data were organized and tabulated t- test was used to compare the mean values of micronuclei and karyolysis cells between the exposed and non-exposed groups (control) using the Statistical Analysis System- SAS program(13).

RESULTS

The results of the current study show that most individuals of the studied sample express different numbers of micronuclei, which appear as a round chromatin mass located in the cytoplasm in vicinity to the nucleus in figure 1.

It can be seen from the data in Table (1) that the mean count of micronucleated cells in traffic policemen was (12.05 ± 0.63). This was slightly greater than the mean count of micronucleated cells calculated in the control group (10.23± 0.84). However the observed difference was statistically not significant (P>0.05). In contrary, the control group showed a slightly higher mean count of karyolytic cells (13.18 ± 1.15) when compared to the exposed group(12.41 ± 0.74)and the difference was statistically non-significant (P>0.05).

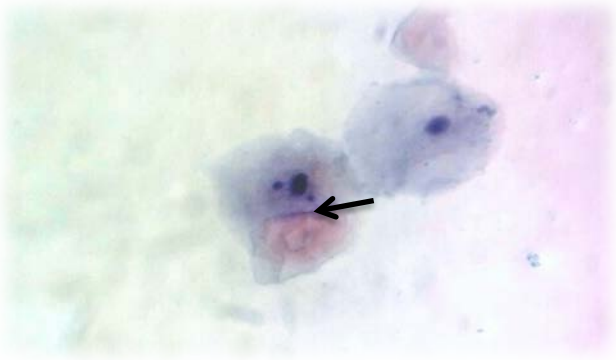

Figure 1: Buccal epithelial cell with two micronuclei (100x)

Table (1) :Mean of micronucleated and Karyolytic cells in buccal cells per 1000 cells of exposed (*n* = 51) and non-exposed (*n* = 22) individuals.

Group	No. of Participants	Mean ± SE	
		Karyolytic cells	Micronucleated cells
Exposed group (traffic policemen)	51	12.41 ± 0.74	12.05 ± 0.63
Control (nonexposed)	22	13.18 ± 1.15	10.23 ± 0.84

Table (2): Mean of micronucleated and Karyolytic cells in buccal cells per 1000 cells of the studied sample according to smoking habits.

Group		Mean ± SE	
		Karyolytic cells	Micronucleated cells
Exposed group (traffic policemen)	Smoking (33)	13.16 ± 0.77	12.16 ± 0.67
	No smoking (18)	11.90 ± 1.02	10.57 ± 0.79
Control	Smoking (10)	13.04 ± 0.80	12.22 ± 0.71
	No smoking (12)	11.78 ± 0.95	10.61 ± 0.82

The distribution of the studied sample according to smoking habits is given in the table (2). Questionnaire findings showed that 33 traffic policemen and 10 controls reporting smokers at the time of the study. Their mean ±SE values of micronucleated cells were 12.16 ± 0.67, 12.22 ± 0.71, whereas the values of karyolytic cells were 13.16 ± 0.77and 13.04 ± 0.80, respectively. There were no significant differences between smokers and nonsmokers in both groups (P> 0.05).

DISCUSSION

The present study was designed to determine the effect of exposure to genotoxic compounds in urban air by using Buccal Micronucleus Cytome (BMCyt) assay. Micronucleus (MN) is a round chromatin mass presents in the cytoplasm nearby the nucleus. It arises in a daughter cell whenever an entire chromosome or a chromosomal fragment fails to be involved in cell division, as shown in figure 1. Micronucleus provides measures of blastogenic and aneuploidy events(7,8).

On the contrary to the expectation our data did not show any significant difference in micronucleated and Karyolytic cells in the exposed group. There are several possible explanations of this result.

Our study included relatively small number of participants which was a mixed sample of smokers and non-smokers, which might made it difficult to detect any effect of exposure to genotoxic compounds in ambient air. Another possible explanation is that using DNA nonspecific stains such as Giemsa has an effect on the detection of micronucleated cells and other nuclear anomalies in buccal epithelial cells in a way that might lead to overestimation of micronuclei(14).

Moreover, the present study failed to illustrate statistically significant differences among smokers and non-smokers with respect to micronuclei frequency and karyolytic cells, both in the exposed groups and in the non-exposed groups. This finding supports the findings of Nersesyan et al.(14) who suggested that tobacco smoke does not increase micronuclei frequency in buccal mucosa cells . Previous results obtained by Celik et al. (15) and Diler and Celik (16) confirm our observation that tobacco smoke lack any significant effects on the frequency of other cytogenetic damage including karyolytic cells in buccal cells of smokers.

Although the results of the current study differ from some published studies carried out in Turkey(17) , Philippines and China (18,19) where researchers found higher MNi frequencies in traffic policemen compared with unexposed controls, they are consistent with Jara-Ettinger(20) who did not find significant differences in the frequency of micronucleated cells nor the other nuclear abnormalities between Mexican welders who are occupationally exposed to welding-fumes and control.

One study that has been conducted in Erbil city (north of Iraq) using chromosomal aberration assay in peripheral blood lymphocytes in this study reported a significant increase in the chromosomal aberrations in lymphocytes of traffic policemen (21). In reviewing the literature no data for the Baghdad population was found. This is the first time that Buccal Micronucleus Cytome (BMCyt)assay is being used to explore the association between exposure to outdoor air pollution and DNA damage in buccal mucosa cells of traffic policemen. Our study will serve as a baseline for future studies.

According to Holland et al. (7) and Bolognesi et al. (22) our results are higher than baseline range of 0.05-1.70 micronuclei ·1000-1 cells. Taken into consideration that all individuals recruited to our study live in Baghdad city, the data reported here appear to support the assumption that all participants are exposed to high level of pollution pressure stemming from the high density of traffic and housing(23).

CONCLUSIONS

In our study the lack of significant differences in the number of micronucleated and karyolytic cells between the exposed and the control groups, revealed that all participants were exposed to same genotoxic agents act with same intensity, and produce a similar cell abnormality. In conclusion, further research studies need to be conducted, including a high number of traffic policemen with the exclusion of smokers, enabling the detection of the effect of exposure to high level of genotoxic and cytotoxic compounds in the air of urban areas.

REFERENCES:-

1- Evans,J.S.; Fnais,M.; Giannadaki,D. and Pozzer,A.2015. The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature 525, 367–371.

2- Bernstein, J.A.; Alexis, N.; Barnes, C.; Bernstein, I.L.; Bernstein, J. A.; NEL, A.; Peden, D.; Diaz Sanchez, D.; Tarlo S.M.and Williams, P.B.2004. Health Effect of air Pollution. Journal of allergy and clinical immunology, 114(5): 1116-1123.

3- Lamia, B.T.; Robert, A. B.; Yann, G.; Béatrice, L. S.; Fatiha, E.; Véronique, B.; Neela, G.; Dana, L. and Kurt, S.2012. Carcinogenicity of diesel-engine and gasoline-engine exhausts and some nitroarenes. the lancet oncology,13(7) :663–664.

4- Nersesyan,A.; Michael Kundib,M; Waldherra,M.; Setayesha,T.; Miroslav Mišíka,M.; Wultscha,G.; Filipicc,M.; Rafael,G.; Barcelosd,M.and Knasmuellera, S.2016.Results of micronucleus assays with individuals who are occupationally and environmentally exposed to mercury, lead and cadmium, Mutat. Res.: Rev. Mutat. Res., http://dx.doi.org/10.1016/j.mrrev.2016.04.002 Contents lists available at ScienceDirect Mutation Research/Reviews in Mutation

Research journal homepage: www.elsevier.com/locate/reviewsmr Community address: www.elsevier.com/locat e/mutres3.

5- Sree Devi, V.; Durga Rao,V.; Hara Gopa,V.; Siva Prasad, B.; Sandhya Devi, G.; Jyothy, A. ; Reddy,P. and Hema Prasad,M. 2009. Cytogenetic evaluation of traffic policemen occupationally exposed 1- to vehicular exhaust. Indian J Med Res 130, 520-525.

6 - Riccardo Crebelli, R.; and Caiola,S.2009. Genetic effects of air pollutants: insights from human biomonitoring studies. Indian J Med Res 130, 501-503.

7- Holland, N. ; Claudia Bolognesi,C. ; Kirsch-Volders,M. ; Stefano Bonassi ,S.;Zeiger,E.; Knasmueller,S. and Fenech,M.2008. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. Mutation Research, 659 , 93–108.

8- Fenech,M.; Holland,N.; Chang, W.P. ; Zeiger,E. and Bonassi, S.1999. The HUman Micro- Nucleus Project—an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans, Mutat. Res. 428, 271–283.

9- Shafi, Farha A. Ali Aqeel M. Ali Al-lami, Rawa Abd Al-Ameer (2018) Micronucleus Frequency in Buccal Cells of Males Exposed to Air Pollution in Kufa City. Al-Mustansiriyah Journal of Science,28(2):43-47.

10- Iraq Population. (2017-11-14). Retrieved 2017-12-01, from http://worldpopulationreview.com/countries/iraq-population/ or

11- Tolbert, P. E.; Shy, C.M. and Allen, J.W. 1992. Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutat. Res.; 271(1) : 69–77.

12- Tolbert, P.E.; Shy, C.M. and Allen, J.W. 1991. Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users. Am. J. Epidemiol., 134(8): 840–850.

13- SAS. Statistical Analysis System, 2012.User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

14- Nersesyan,A. ; Kundi,M ; Atefie,K. ;Schulte-Hermann,R. and Siegfried Knasmüller,S. 2006.Effect of Staining Procedures on the Results of Micronucleus Assays with Exfoliated Oral Mucosa CellsCancer Epidemiol Biomarkers Prev . 15 (10) 1835-1840.

15- Celik, A.; Cavas,T. Ergene-Guzokara,S. 2003. Cytogenetic biomonitoring in petrol station attendants: micronucleus test in exfoliated buccal cells. Mutagenesis 18: 417-421.

16- Diler, S.B.and Celik. A. 2011. Cytogenetic biomonitoring of carpet fabric workers using micronucleus frequency, nuclear changes, and the calculation of risk assessment by repair index in exfoliated mucosa cells. DNA and Cell Biology 30: 821-827.

17- Karahalil, B.; Karakaya, A. E. and Burgaz, S. 1999. The micronucleus assay in exfoliated buccal cells: application to occupational exposure to polycyclic aromatic hydrocarbons. Mutat. Res., 442, 29–35

18- Hallare, A. V.; Gervasio, M. K.; Gervasio, P. L. and Acacio-Claro, P. J. 2009. Monitoring genotoxicity among gasoline station attendants and traffic enforcers in the City of Manila using the micronucleus assay with exfoliated epithelial cells. Environ. Monit. Assess., 156, 331–341.

19- Zhao, X., Niu, J., Wang, Y., Yan, C., Wang, X. and Wang, J. (1998) Genotoxicity and chronic health effects of automobile exhaust: a study on the traffic policemen in the city of Lanzhou. Mutat. Res., 415, 185–190.

20- Jara-Ettinger, A.C.; López-Tavera, J.C.; Zavala-Cerna, M.G.and Torres-Bugarín, O. 2015. Genotoxic Evaluation of Mexican Welders Occupationally Exposed to Welding-Fumes Using the Micronucleus Test on Exfoliated Oral Mucosa Cells: A Cross-Sectional, Case-Control Study. PLoS ONE, 10(8): 0131548. doi:10.1371/journal.pone.0131548

21- Kazhal, M. S.2016. Cytogenetic study of traffic policemen occupationally exposed to vehicle Exhaust in Erbil City/ Iraqi Kurd-istan Region. Iraqi Journal of Cancer and Medical Genetics, 9 (2) 187-194.

22- Bolognesi, C.; Knasmueller, S.; ;Nersesyan, A. ;Thomas, P.and Fenech,M. 2013. The HUMNxl scoring criteria for different cell types and nuclear anomalies in the buccal micronucleus cytome assay – an update and expanded photogallery. Mutation Researh, 753: 100-13.

23- Shafi ,F.A.A; AL-Ansari ,N.and Abdul Majeed, B.A. 2016. Frequency of micronuclei in peripheral blood lymphocytes of healthy individuals: A study from Baghdad, Iraq. World J Exp Biosci 4: 40-48.