Journal of Chemical Health Risks 2(3); 1- 4, 2012
ISSN:2251-6719

The Effects of Environmental Pollution with Heavy Metals in Frequency of Micronuclei in Epithelial Buccal Cells of Human Population in Mitrovica

K. Letaj, I. Elezaj, Q. Selimi and K. Kurteshi

Department of Biology, University of Prishtina,
P.O.Box 10.000 Prishtinë, Republic of Kosovo

Abstract: The purpose of this study was evaluation of genotoxic effects of environmental pollution with heavy metals in inhabitants of Mitrovica town (which is located close to smelter "Treça" down closed). In this study, 55 inhabitants (males) from Mitrovica town and 20 (males) control subjects with similar mean ages and smoking prevalence were enrolled for analysis of micronuclei frequency in epithelial buccal cells. The subjects of Mitrovica town showed significant increase micronuclei frequency ($p< 0.001$) in epithelial buccal cells compared to controls and with respect to their smoking habits. The current study suggests that chronic exposure to pollution with heavy metals could lead to increase of DNA damage.

Key words: micronuclei, DNA, buccal cells, heavy metals

INTRODUCTION

Over the 30 years a large literature has been published on the epidemiology and mechanistic toxicology of the heavy metals and metalloids, lead, cadmium, arsenic and mercury. They are globally recognized as important health risk associated with occupational and environmental exposure. More recently, research has been initiated on interactions between these toxic elements and genetic susceptibility factors that could influence individual responses to metal exposure. The metal exposures are usually complex and most populations are exposed to more than on metal at a time (1).

One of the most rapid and efficient techniques to study the impact of environmental and life –style factors on genetic stability in human populations is the micronucleus (MN) test (2). MN is formed by centric chromosomal fragments or whole chromosomes that are not includes in the main daughter nuclei during nuclear division. MN induction therefore reflects clastogenic and/ or eugenic events (3). Nevertheless, about 92% of human cancers are derived from epithelial tissue. Micronucleus test in exfoliate buccal cells has been shown to be an effective method to detect unstable chromosomal aberrations (4).

The city Mitrocica is located between a lead smelter ("Trepça") and refinery in north, and a battery factory, a zinc electrolysis plant, a sulfuric acid plant, and a super phosphate fertilizer industry in south. The most abundant air pollutant in the region has been lead dust. The concentration of lead in air has been measured monthly in five localities in the town since 1973. The mean annual air-lead concentrations in these locations ranged from 7.8 to21.7 $\mu g/m^3$ in 1973. By 1980, the air-lead concentrations head increased to 21.3-29.9 $\mu g/m^3$. At that time in ex-Yugoslavia, the maximal allowable concentration of lead in air has been 0.7 $\mu g/m^3$; at times, the air lead concentration has reached 100 times this allowable limit (6).

Environmental exposure to lead in children remains an obvious and important public health problem in the Republic of Kososvo, especially in urban and

Lead and inorganic lead compounds are classified by International Agency for Research on Cancer (IARC) in group 2B as possibly carcinogenic to humans (IARC, 1980). Lead is mostly negative in vitro assays of genetic toxicology, although at high levels lead clearly crosslink's proteins and DNA, binds to the phosphate groups of DNA and changes its conformation (5). Lead, like other heavy metals, can cause cytogenetic damage with induction of micronuclei (MN), but the mechanism of this phenomenon is unknown.

TADY AREA, MATERIAL AND METHODS

The city of Mitrovica, approximately 40 km north of Prishtina, the capital of The Republic of Kosovo, has been the site of one the largest lead smelters in Europe. Lead-related industries have been a major element of the economy of Kosovo before the war, but created extensive health risk due to environmental pollution with lead and a variety of other substances. Approximately 200.000 people has been exposed to lead emissions from lead smelter, 100.000 of whom lived in the highly exposed city area which lies at the convergence of Lumi i Bardhë and Sitnica rivers.

Corresponding Authors: K.Letaj, Department of Biology, University of Prishtina. P.O. Box 10.000 Prishtinë, Republik of Kosovo

industrial regions. Locations where the lead ores are extracted and processed, in particular the city of Mitrovica with its surroundings, represent specific area of concern. Gradually decreasing levels of lead emissions (in August , 2000 year the "Trepça" smelter was closed down by NATO troops) and declining air lead concentrations are not followed by immediate elimination of potential health hazards related to lead exposure, such as neurodevelopment deficiencies, adverse effects on the haemopoietic system and genotoxic risk. Buccal cell samples were obtained from 55 subjects (males) who lived in polluted area with heavy metals (Mitrovica town) and in the control individuals (20 males) from Çitak village-rural area. Exfoliated buccal cells were scraped gently with a cytobrush and the material was submersed in 5 ml of saline solution (0.9% NaCl). After centrifugation (10 min at 1200 rpm), the pellet was fixed in 3:1 methanol/acetic-acid once, or twice if necessary, for 5 min. Slides were stained with May-Grunwald-Giemsa according to standard protocol (4). Exfoliated buccal cells were analyzed under a total magnification of x1000 using a Leica microscope with immersion. The following criteria for MN analysis were used in oral exfoliated cells. MN must: a) be less than 1/3 diameter of the main nucleus, b) be on the same plane of focus, c) have the same color, texture and refraction as the main nucleus, d) have smooth oval or round shape, and e) be clearly separated from the main nucleus. Two slides were prepared for each subject and 2000 cells from each subject were examined, and results were reported as the frequency of cells with micronucleus. Student's t test was applied to determine the statistical significance.

micronuclei in exfoliated buccal cells exposed group to heavy metal pollution, and control group are presented in Table. 1.

RESULTS

The main demographic characteristics (age/years, years of work, and years of smoking, cigarettes/day) and results of frequency of

Table1. General characteristics and frequency of micronuclei in exfoliated buccal cells of subjects exposed to heavy metal pollution and none exposed subjects

	Number of subjects	Age/year	Years of work	Years of smoking	Cigarettes/day	Frequency of MN/2000 cells
Control Group						
Non-smokers	18	40.0±16	0.4 ± 1.3	-	-	1.5±0.8
Range		(19-64)	(3-10)			(1-3)
(A)						
Smokers	2	57.5±19	22.5±17.6	10±0.1	7.5±3.53	5.0±0.1**
Range		(44-71)	(10-35)	(10)	(5-10)	(3-5)
(B)						
Total	20	41.7±16	2.6±8.0	-	-	1.8±1.3
Range		(19-71)	(3-35)			(1-5)
(I)						
Exposed Group						
Non-smokers	47	30.8±17	3.4±7.1	-	-	6.1±3.8***
Range		(16-70)	(1-20)			(1-10)
(C)						
Smokers	8	49.5±7.4	13.6±7.5	16.5±9.0	24.6±18.6	12.7±1.9***
Range		(40-63)	(9-30)	(4-30)	(5-60)	(10-14)
(D)						
Total	55	33.5±17	4.8±7.9	-	-	7.0±4.02***
Range		(16-70)	(1-30)			(1-14)
(II)						

Note: The results are expressed as means ± SD; ** P <0.01; *** P <0.001.

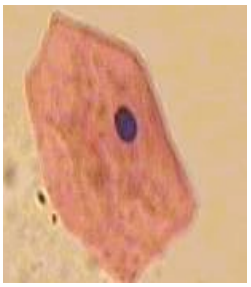
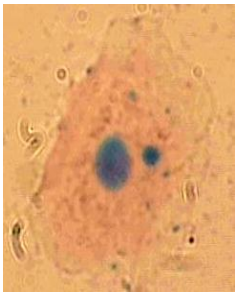
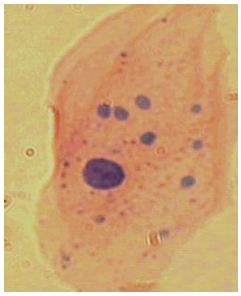

Fig.1 Fig.2 Fig.3

Figures 1 -3. Epithelial buccal cells without, with one and more micronuclei

Our results presented in Table 1 show significantly higher (P <0.001), frequency of MN in epithelial buccal cells of subjects from Mitrovica town in comparison with frequency of MN of control subjects. Additionally, the MN frequency of epithelial buccal cells of smoker subjects from Mitrovica was significantly higher (P < 0.001), in comparison with MN frequency of non-smoker subjects from the same area. On the other hand the MN frequency of epithelial buccal cells of smokers from control subjects were also significantly higher (P <0.001) in comparison with MN frequency in non-smoker subjects of controls.

DISCUSSION

ur results of higher frequency of MN fin epithelial buccal cells of subjects from Mitrovica (heavy metal polluted area), are in accordance with results of (7) who in epithelial buccal cells of male battery workers (64 workers) confirmed significant increase of micronuclei. The present results are in accordance with results of (8), who reported significant increase of micronuclei in peripheral lymphocytes of 103 lead exposed workers. Our results are also consisted with the results of Hamurcu et al. (9) who found high frequency of micronuclei in peripheral blood lymphocytes of workers exposed to Pub, Zn, and Cd . On the other hand Martino-Roth et al. (10) , also recorded genotoxic effects in batteries and garage car workers. The results of higher frequency of MN of Mitrovica subjects are consistent with results of Letaj et al. (11), who reported significant increase of micronuclei in peripheral lymphocytes of house cats (Felis domesticus) from Mitrovica town. Our results of higher frequency of micronuclei of epithelial buccal cells of smoker subjects not only from Mtirovica but also in control subjects are in accordance with results of Çelik et al. (12) who reported that cigarette smoking significantly increased the rate of DNA damage such as incidence of micronuclei in humans. Workers in many occupational settings and environmental pollution conditions with heavy metals are exposed to mixture of genotoxic agents. These people may not be aware that they have been exposed to genotoxic agents nor do they know the type and amount of agent they have been exposed. Therefore, there is a need to educate those who work with heavy meats or live in environments industrially polluted with heavy metals about the potential hazard of occupational and environmental pollution exposure and the importance of using protective measures. The habit of smoking enhanced the frequency of micronuclei in buccal cells when compared with controls. Buccal epithelium cells provide an alternative source of tissue in human subject's monitoring to occupational and environmental toxic exposures. The current study suggests that chronic exposure to pollution with heavy metals could lead to increase of DNA damage.

REFERENCES

1.Silbergeld E.K. 2008. Gene: Environmental interactions in metal toxicity. Proceedings of 14th International Conference on Heavy Metals in the Environment. 273-275.

2.Nersesyan A.K., Vardazaryan N.S., Gevorgyan A.L. Arutyunyan R.M. 2002. Micronucleus level in exfoliated buccal murosa cells of kancer patients. Archive of oncology. 10: 35-36.

3.Fenech M., Holland N., Chang W.P., Zeiger E., Bonassi S. 1999. The Human MicroNucleus Project- an internacional collaborative study on the use of the micronucleus technique for measuring DNA damave in humans. Mutation Research.. 428:271 283.

4.Tolbert,P.E., Shy,C.M. and Allen,J.W. 1992. Micronuclei and other nuclear bnormalities in buccal smears: methods and development. Mutat. Res.,271, 69-77.

5.Grandjean P., Wulf H., Nebuhr. 1983. Sister chromatide Exchange in lead-exposed worcers,Environ Res. 199-204.

6.Popovac D, Graziano J, Seaman C, Kaul B, Colakovic B, Popovac T., Osmani, I., Haxhiu, M., Begraca, M, Bozovic Z, Mikic M. 1982. Evaluated blood lead in a population near a lead smelter in Kosovo, Yugoslavia. Arch. Environ. Health, 37, 19-23.

7.Devi K.R., Madhavi D., Kumari J.K. 2011. Nuclear anomalies in exfoliated buccal cells od occupationaly lead exposed population. International Journal of Pharma and Bio Sciences. 2. 4: B 710-B712.
8.Alexander A.C., Sonia S.P. Rchardo M. 2001. Occupational exposure to lead and induction of geentic damave. Environ Health Per. 2001; 109 (3): 295-298.
9.Hamurcu Z., Donmez H., Saraymen R. and Demirtas H. 2001. Micronucleus Frequencies in workers Exposed to Lead, Zinc and Cadmium. Biological Trace Element Reserch.; 83. 97-102
10.Martino-Roth M.G., Viégas J., Amaral M., Oliveira L., Ferreira F. L. S and Erdtmann B. 2002. Evaluation of genotoxicity through micronuclei test in workers of car and battery repair garages. Genetics and Molecular Biology, (25); 495-500.
11.Letaj K.Rr., Elezaj I.R., Selimi Q.I. , Kurteshi K.H. 2011. The Genotoxic Effects of Environmental Pollution with Heavy Metals in cat (Fesils domesticus). Proceedings of 15th International Conference on Heavy Metals in the Environment. 661.
12.Çelik, A., Çavaş, T. and Ergene-Gözükara, S. 2003. Cytogenetic Biomonitoring in petrol stacion attention Micronucleus test in exfoliated buccal cells. Mutagenesis. 2003; 18: 41—421.
13.International Agency For Reserch on Cancer (IARC) 1980. Monographs on the Evaluation of the Carcinogenic Risc to Humans-Some Metals and metallic Compounds, WHO, Lyon, 23,; 1-210,325-415.