ISSN 0975-6299 Vol 2/Issue 4/Oct – Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

CYTOLOGY

NUCLEAR ANOMOLIES IN EXFOLIATED BUCCAL CELLS OF OCCUPATIONALLY LEAD EXPOSED POPULATION

K.RUDRAMA DEVI*, D.MADHAVI AND J.KARUNA KUMARI.

Human Genetics Lab, Department of Zoology, Osmania University,Hyderabad.

K.RUDRAMA DEVI
Human Genetics Lab, Department of Zoology, Osmania University,Hyderabad.

ABSTRACT

Lead (Pb), a toxic contaminant metal used in many important industrial processes, is widely used in batteries, paint vanishes, in pipe covering and in welding. The exposure showed a high risk of human health and development of several types of cancers. A total of 64 male workers in Hyderabad battery unit in Mahaboobnagar District, Andhra Pradesh were investigated for genetic damage in buccal cells of exposed and control subjects. A questionnaire based survey was conducted and buccal smears were conducted from oral cavity and analyzed for nuclear anomalies. A high frequency of Karyolysis was observed among male workers. The percentage of nuclear anomalies was observed in workers inhaled lead oxide fumes in Industrial atmosphere. The habit of smoking enhanced the frequency of micronuclei in buccal cells when compared with controls. The present study clearly reveals the mutagenic nature in occupational exposure to lead.

KEY WORDS

Lead battery unit, Micronuclei, buccal cells,Genotoxicity

INTRODUCTION

Lead is widely used in industry and disseminated via air, Industrial Pollution, agricultural technology and processing (Schuller and Egan 1976). Numerous studies have shown that 15 to 30% lead exposure for general population comes from inhalation and 70 and 80% from ingestion (Eari et.al. 1999).Consequently all individually have a body burden of lead, whether they are exposed in urban, rural or occupational environments. The average whole blood lead level of healthy adults in the USA has been reported 0.72 to 1.93 and is slightly higher in urban population (Goldwater 1967, Eari et.al. 1999).

Lead is commonly found in home and industrial surroundings and causes a variety of adverse health effects (Zou et al.2003).Lead has been a known toxicant for thousands of years, and it remains a persistent environmental health threat. Exposure to lead can result in significant adverse health effects in multiple organ systems. Toxic effects on the nervous, hematologic, renal and reproductive systems have been studied extensively and are well documented (Goyer 1996, Mahaffey 2000). Additionally, living in close proximity to lead-emitting industrial facilities can present a significant source of cumulative exposure to lead via air, water and soil. Occupational exposure to lead is most often encountered at lead smelters and battery manufacturing facilities, as well as in house renovation projects in which workers inhale and ingest lead –contaminated fumes and dust from lead based paint.

A quantitative assessment of published data with workers heavily exposed to inorganic lead provides some evidence to support the hypothesis of an association between stomach and lung cancer (Fu et.al. 1995). But this Meta analysis is limited significantly by the lack of information on potential confounding factors.

Adverse effects of lead at low levels of exposure especially crucial stages of brain development. The occupational exposure to these solvents was typically found in workers of rubber, pharmaceuticals, paint and pigment and shoe making industries. The exposure of organic solvents can lead to euphoria and hallucinations while high doses may produce life threatening effects such as convulsions and coma. The long term exposure may lead to cancer risk (Fu et.al. 1996). Nevertheless about 93% of human cancers are derived from the external and internal epithelium. On the other hand, the micronucleus test in exfoliated epithelial cells has been shown to be effective method to detect unstable chromosomal aberrations. (Kalya et.al. 1993, Pastor et.al. 2001). Buccal epithelium cells provide an alternative source of tissue in human subject's monitoring for occupational and environmental toxic exposures. Hence the objective of the present investigation was to study the extent of cytogenetic damage in exfoliated buccal cells obtained from lead battery unit workers. Degenerative nuclear changes, such as micronuclei (MN) binuclei(BN) Karyorrhexis (KR) and Karyolysis (KL) were analysed in the exfoliated buccal cells.

MATERIALS AND METHODS

(i)Study Population

The study was carried out in 62 lead battery factory workers. The control group consists of 60 healthy individuals with no exposure to any toxicant or any other chemicals. Participants are informed about the study, asked to sign the consent form and complete the questionnaires to obtain

necessary information on their life style and personal habits (age, working duration, smoking habits, health etc.).The protocol adapted in the present study is approved by Institutional Ethical Committee ,Osmania University,Hyderabad.

(ii)Preparation of Buccal Cell Sampling:

Prior to buccal cell collection the shoe factory workers were advised to rinse their mouth thoroughly with water to remove unwanted debris. Sterile wooden spatula was used to obtain cell samples from buccal mucosa. The mucosa was transfered to Eppendoff tubes with PBS with P^H.7.0 and centrifuged for 10 min at 1500 rpm. Supernatant was removed and replaced with fresh PBS solution. This process was repeated thrice and the pellet was smeared on clean slides. Smears were air dried and fixed in 1:3 acetic acid and methanol fixative for 10 min. Slides are air dried and stained with 2% Giemsa for 10 Min. The slides are air dried and observed under microscope.

Scoring criteria for buccal cytome assay. From each sample three slides were scored, and nuclear abnormalities were classified according to the Tolbert et.al. (1992). These criteria are intended to classify buccal cells into categories that distinguish between “Normal and Abnormal” based on their aberrant nuclear morphology. The abnormal morphologies are due to the DNA damage and cell death.

(iii)Scoring Method

Micronuclei are identified with the presence of main nucleus and one or more smaller nuclei (micronuclei) in cells. The micronuclei are usually round or oval in shape and their diameter may range between 1/3 to 1/16 the diameter of main nucleus. Binucleated cells have two nuclei that are adherent to each other. This is indicative of failed cytokinesis. Karyorrhectic cells have dense network of nucleochromatin elements that lead to fragmentation and disintegration of the nucleus. In Karyolytic cells, the nucleus is devoid of DNA and appears to have no nuclei. This indicates a very late stage of cell death process. It has a cloudy appearance with no distinct features (Figure 1,2,3and 4).

(iv)Statistical Analysis.

To determine the frequency of various cell types, about 1000 cells were scored for the presence of micronuclei cell, binucleated cells, Karyorrhectic and Karyolytic cells. All the data were expressed as the Mean Standard Deviation. The synergistic effect between smoking and exposure were tested with a two way analysis of variance. Multiple comparisons were made by using a least significant difference test. The error rate was accepted as 0.05 by student + test.

RESULTS AND DISCUSSION

Table 1 and 2 shows the main characteristics in controls – cases studied. The mean age group of the selected workers belongs to the range from 32.4 $\pm$ 6.2 to 42.2 $\pm$ 8.0 in control group and from 38.0 $\pm$ 7.0 and 40.2 $\pm$ 8.0 in the exposed group. They belonged to the similar social economic status. The characteristics of the studied group are mentioned in Table 1. The cytological observations reveal micronuclei and binucleated cells of buccal smears. The mean value of micronuclei in smokers was 10.12 $\pm$ 1.01 as against 8.20 $\pm$ 2.12 in non smoker exposed group. The mean value of binucleated cells in mean subjects without smoking was 18.80 $\pm$ 1.03 as against 46.00 $\pm$ 1.00 in subjects with a habit of smoking higher cells of Karyorrhexis cells (KRC) and Karyolytic Cells (KLC) mean were significantly higher in smokers of exposed subjects compared to non smokers exposed group. This indicates that the habit of smoking enhanced the mean values of KRC and KLC nuclear anomalies when compared to control values. The frequency of mcironucleate cells, binucleate cells, Karyorrhexis and Karyolytic cells were compared in duration of exposure less than 5 years and in ten years exposure and it is more

signficantly higher in ten years of service workers. Table 2 and 3.

The harmful effect of dust in various forms of human health have already been demonstrated (Gutherie 1992, Dong et.al. 2006, Prince et.al. 2008). The MN Test, that is scientifically approved, is important in demonstrating the genotoxic effects of harmful substances on health (Nerseyan, 2005, Fenech et.al. 2007) such as measuring genotoxicity in petrol station employees (Celik 2003, Benities et.al. 2006) agricultural workers (Pastor et.al) Cigarette smokers and tobacco users (Kul et.al. 1997, Prioita et.al. 2006) workers exposed to pesticides (Pastor et.al. 2002) Polycyclic Hydrocarbons (Karahalil et.al. 1999) timber dust (Celik and Kanik 2006) Ozone and Cancer patients (Chen et.al. 2006 Bloching et.al. 2000).

The Micronuclei in exfoliated epithelial cells are useful biomarkers of occupational exposure to genotoxic chemicals. Cigarette smoking is one of the factors that may influence the rate of DNA damage such as incidence of micronuclei in humans (Celik et.al. 2003). It is reported that cigarette smoking significantly increased the frequencies of nuclear abnormalities in both controls and exposed subjects. Increase in exposure to toxic chemicals such as formaldehyde and benzene induces a significant increase in the buccal cell micronuclei (Titenko Holland et.al. 1996, Suralles et.al. 1997) (Lewinska et.al 2007). The present results are comparable with that Alexander et.al. (2001) who reported significant increase in micronuclei in peripheral lymphocytes of 103 lead exposed workers. Further in another study Chen et. al. 2006 showed higher incidence of micronuclei in buccal cells of lead battery units.

The present results are comparable with our earlier studies such as increased frequencies of micronuclei in industrial painters (Madhavi 2008) and in shoe factory workers (Jitender Naik et al.2005).In summary, this study shows a clear genotoxic effect associated with occupational exposure to lead .These data are relevant and permit an estimate of genetic risk of lead by using biomarkers of exposure.

The micronucleus assay in human exfoliated cells is one of the most sensitive methods used for measuring DNA damage rates in human populations; because it is relatively easier to score micronucleus compared to other methods, such as chromosome aberrations. This assay can be used to identify not only groups that are at risk for developing cancer, but also specific individuals who are susceptible to cancer development.

Our results make it clear that lead exposed workers showed an increased frequency of Cells with micronuclei, due to the genotoxic effect of the petroleum derivates to which they are exposed.

Extensive studies and standardized tests to evaluate biological damage at different levels are recommended to public agencies concerned with environmental quality and public health. Mutagenic investigation is one of the necessary evaluations to be done, to ensure environmental quality and occupational health, as is the worker's education about decreasing genetic damage and risk for serious diseases.

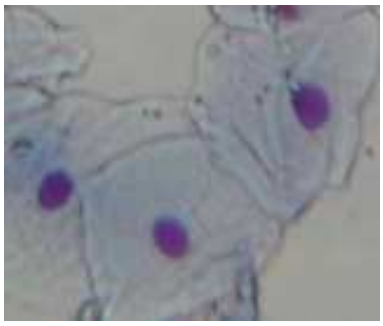

Figure:1
Cell without Micronuclei

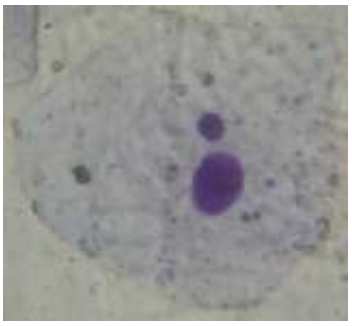

Figure: 2
Cell with micronuclei

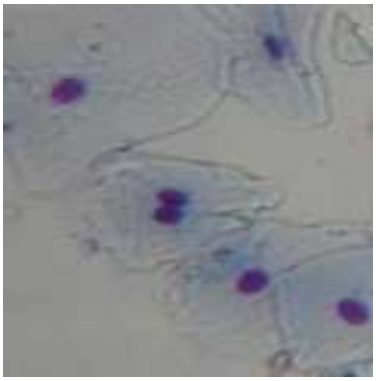

Figure : 3
Binucleated cell

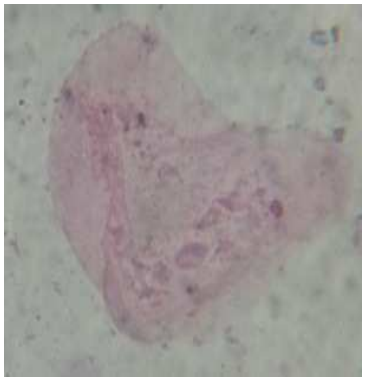

Figure : 4
Karyolytic cell

Table – 1
Demographic Characteristics of case control Study.

	Control		Exposed Group	
No. of Samples	Number	Age	Number	Age
Smokers	28	28±o.10	30	31.92±1.60
Non-smokers	32	31.0±0.11	32	35.10±1.02

Table – 2
Cytological observation in control groups.

Individuals	MNC	BNC	KRC	KLC
Smokers	3.60 ± 0.10	3.72 ± 0.40	8.62 ± 0.40	32.40 ± 0.60
Non Smokers	2.10 ± 0.02	3.10 ± 1.20	6.10 ± 1.20	26.42 ± 1.01
Age				
<25	2.42 ± 0.08	4.20 ± 0.48	9.12 ± 1.08	28.10 ± 1.08
>25	2.70 ± 0.10	2.92 ± 0.68	6.80 ± 0.41	22.70 ± 0.12

Data are reported as mean + SD
*P<*0.05*

Table – 3
Cytological observation in Subjects

Individuals	MNC	BNC	KRC	KLC
Smokers	10.12	6.82	18.80	46.00
Non Smokers	08.20	4.60	08.20	31.00
Age				
<25	08.72	6.72	14.12	22.54
>25	10.60	8.10	18.20	28.04
Duration of Exposure				
<5 years	6.12	5.10	18.80	30.14
>5 years	9.10	7.82	22.40	38.10

Data are reported as mean + SD
**** P Value <0.05 significant level.***

ACKNOWLEDGMENTS

The research work is funded by DRDO LSRB Minstry of Defence, Govt.of India. We thank Dr.Ilavazhagan, Director, DIPAS for valuable suggestions while preparing the manuscript .

REFERENCES

1. Alexander A made C, Stoyan Sonia P and Richardo M, Occupational exposure to lead and induction of genetic damage. Environ heal per V. 109(3) 295 – 298 (2001).
2. Bloching M,Hofmann A,Lautenschlager C, Berghaus A and Grummt T Exfoliative cytology buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN Oncol 36:550-555. (2000)
3. Benites CI, Amado LL, Vianna RAP and Martion – Roth MG Micronucleus test on gas station *Genet Mol* Res. 5:45-54. (2006)
4. Chen C, Arjomandi A, Qin H, Balmes J, Tager I and Holland N Cytogenetic damage in buck and peripheral lymphocytes of young healthy individuals exposed to ozone. Mutagenesis 21:131. (2006)
5. Celik A, Cava T and Ergene Gozukara S Cytogenetic Biomonitoring in petrol station attention Micronucleus test in exfoliated buccal cells. *Mutagenesis* 18: 417 – 421. (2003)
6. Celik A, Kanik A Genotoxicity of Occupational Exposure to wood dust: Micronucleus frequency nuclear changes in exfoliated buccal cells. Environ Mol Mutagen 47 (9) 693 – 698 (2006).
7. Dong F, Deng J, Pu ZX and John H Pulmonary alveolar macrophage cytotoxicity investigation shape mineral dusts. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi 23 848 – 51. (2006)
8. Eari BD, Douglas RE, William A, Harris BS Melvin C, Tester BS, William J. MC Granity MD. The effect of AA supplementation on blood lead levels of smokers J. Ameri Coll Nutri 18(3) : 166-170 (1999).
9. Fu H Boffetta P Cancer and occupational exposure to inorganic lead compounds : a metanalysis of published data Occup Env. Med 52 : 73-81 (1995).
10. Fenech M. Bolognesi C, Kirsch – Volders M, Bonassi S, Zeiger E, Knasmuller S and Holland N Harmonistation of the micronucleus assay in human buccal cells

– A Human Micronucleus (HUMN) initiative commencing in 2007. *Mutagenesis* 22:3-4. (2001)
11. Goyer,R.A. Toxic effects of metals.In: Klaassen C.D.ed.Casarett and Doull's Toxicology :The Basic Science of Poisons,5th ed.,McGraw-Hill Book Company, New York,pp.691-736. (1996).
12. Guthrie Jr GD Biological effects of inhaled minerals. *Am Mineral* 77: 225 – 243. (1992)
13. Goldwater LJ, Hoover AW. An international study of "normal" levels of lead in blood and urine. Arch Envir Health 15 : 60-63 (1967).
14. Jithender Kumar Naik,S.,Rudrama Devi,K.Vasundara Devi and Molly Krupavaram.Analysis of Micronuclei (BNMN)in peripheral blood lymphocytes of leather shoe-factory workers exposed to organic solvents –A case study.Indian J.Environ.Toxicol., 15(1):23-25.(2005)
15. Karahalil B, Karakaya AE and Burzaz S the Micronucleus assay in exfoliated buccal cell s: occupational exposure to polycyclic aromatic hydrocarbons. *Genet Toxicol Envion Mutagen* 442 : (1999)
16. Kalyanaswamy B, Rudrama Devi and Kaneshewari M. Genotoxic effects of heavy metals in mammals 3 Effect of lead nitrate on germ cells of mic. Ind. J. of Env. Biology 14(3) : 249-254 (1993).
17. Levinenska AJ, Savian A, Talaksa G, Boeniger MF, Suruda A and Schulte PA the Utility of epit Micronuclei in the assessment of intermittent exposure. *Biomarkers* 2:135-138. (1997)
18. Madhavi, Cytogenetic and Biochemical Studies in Painters.Ph.D.Thesis.(2008)
19. Mahaffey,K.R.,McKinney,J.,Reigart,J.R.2000.Lead and compounds.In:LippmannM,ed.Environmental Toxicants,Human Exposures and Their Health Effects,2nded.,JohnWiley and sons,Inc.,New York,481-521. (2008).
20. Nersesyan AK Nuclear buds in exfoliated human cells. *Genet Toxicol Environ Mutagen* 88. (2005)
21. Pastor S, Creus A, Xamena N, Siffel C and Marcos R Occupational exposure to pesticides damage : Results of a Hungarian population study using the micronucleus assay in lymphocytes *Envrion Mol Mutagen* 40 : 101 -109. (2002)
22. Prince AP, Kleiber PD, Grassian VH and Young MA reactive uptake of acetic acid on calcite reacted calcite aerosol in an environmental reaction cham
23. Prioia NK,Paszkiewicz GM,Nasca MSS,Franke GE and Pauly JI(2006) Smoking and smokeless t associated human buccal cell mutations and their association with oral cancer- A review.Cancer Biomarkers Prevent 15:1061-1077.
24. Schuller PL, Egan H Cadmium, lead, mercury and methyl mercury compounds. A review of methods of trace analysis and sampling with reference to food. Food and Agriculture Organisation of USA 29-57 (1976).
25. Titenko – Holland N, Moore LE, Smith MT Measurement and characterization of micronuclei in exfoliated human cells by fluorescence in Situ hybridization with a centrometric probe. *Mut Res.* 313:39-50. (1994).
26. Tolbert PE, Shy CM and Allen JW Micronuclei and other nuclear anomalies in buccal smear development. *Mutat Res*.271:69-77. (1992)
27. Zou,C.,Zhao,Z.,Tang,L.,Chen,Z .and Du,L..Effects of lead on brain stem auditory evoked potentials in children.J.Chin.Med.,116:565-568. (2003)