

Evaluation Of Genetic Damage In Buccal Epithelial Cells Of Asbestos Industry Workers Using Micronucleus Assay

Rudrama Devi.K.

Karuna Kumari. Jaddu.

Human Genetics and Toxicology Laboratory,
Department of Zoology, Osmania University, Hyderabad,
Telangana, India

Abstract: Asbestos is known carcinogen. Workers employed in asbestos industry are exposed to larger amounts of cement dust particles in work place which leads to harmful health effects. In the present study assessment of genetic damage was evaluated in buccal epithelial cells of asbestos industry workers using micronucleus assay. A total of 88 subjects were selected and 66 individuals were served as controls having same diet and social economic background. The buccal cells were collected from oral cavity and analysed for nuclear abnormalities. A high frequency of karyolysis was noted in exposed group with longer duration of exposure. A significant increase in the frequency of micronucleus was observed in exposed group when compared with control data. A synergistic effect was observed in smokers of exposed group. Thus the data clearly indicates the ill effect of cement dust in asbestos industry workers and the present studies reveals the use of micronucleus assay to identify the groups that are at risk for developing cancer.

I. INTRODUCTION

Populations of industrial areas are intensely exposed to chemical substances that can cause mutations, cancer, and congenital defects. Cement industries discharge cement dust into the environment from various points of the production process such as the crusher, rotary kiln, cranes, industries, storage silos, and packing sections (Dedobbeler 1991). Increasing amounts of potentially harmful particles are being emitted into the workplace atmosphere, which results in human health effects. Recent epidemiological surveys relate 2-8% of all cancer is due to exposure to carcinogens at the workplace and some studies have indicated that cement and concrete constituents might be carcinogenic. A case-control study in Denmark found that people working in concrete and cement manufacturing had an increased risk of laryngeal cancer (Olsen et al., 1984) and a Swedish cohort study reported an increased risk of colorectal cancer in cement exposed men (Jakobsson et al., 1993). An increased risk of lung cancer, gastrointestinal tumours and dermatitis were also reported in diverse studies (Jakobsson et al., 1993; Yang et al.

1996; Abu Dhaise et al., 1997; Algranti et al., 2001; AlNeaimi et al., 2001; Stern et al., 2001). Cement dust contains mixture of calcium oxide, silicon oxide, aluminium-tri-oxide, ferric oxide, magnesium oxide, clay, shale, sand and other impurities. The cement dust particles mainly enters into the body through respiratory and gastrointestinal tracts (Maciejewska 1991, Abou et al 1995). It has also been reported that cement dust particles could be found in various body organs including liver, spleen, bone and blood and they produce different type of lesions.

Cement dust exposure occurs commonly in the cement production and construction industry. Most studies carried out in these industries, however, focus on silica exposure and on cement dust exposures in cement plants only inadequate data is available. There is no data published about genotoxicity among construction workers. Millions of workers in India are potentially exposed each year to hazardous chemicals, dusts, or fibers in construction settings. Some of these agents are genotoxic and may cause genetic alterations in the somatic or germ cells of exposed workers. Such alterations may lead to the development of cancer. The risk of cancer is less easy to

detect with traditional epidemiological methods in the construction industry than in other industrial sectors. It is not sufficient to rely upon broad epidemiological data to estimate the risk of cancer due chemicals in the construction industry (Jarvholm, 2006).

Asbestos is a known carcinogen and its carcinogenic properties has been reported (Mossmar and Gee, 1989) epidemiological and clinical studies have shown asbestos fibres are associated with development of neoplasia, lung cancer, malignant mesothelium (Jaurand 1997, Manning et al., 2002). Mineral and man made vitreous fibres are used for hundreds of years for several purposes. Asbestos fibres are extensively used for various industrial purposes. Asbestos is used for manufacturing asbestos – cement – sheets, asbestos – cement pipes brake lining, clutch lining, asbestos yarns ropes gaskets k seals etc. Mostly asbestos industrial units use chrysotile variety of asbestos. However the mutagenic effects are controversial (Champlon M and Tahnuay 1977, Reiss et al 1982, Lavappa et al 1974) text found to mutagenic (Kalyan swamy et al 1993, Laxman Rao et al 1987) in the present investigation the genetic damage was assessed in asbestos industry workers exposed to cement dust using micronucleus assay in buccal epithelial cells.

II. MATERIALS AND METHODS

A. SUBJECT RECRUITMENT AND SAMPLE COLLECTION

The study was carried out in 108 asbestos exposed workers aged 16- 60 years from Telangana region of India. The control group consisted of 88 healthy individuals with no exposure to any toxicant or any other chemicals and socio-economic status is also similar to that of experimental subjects. At the time of sample collection all the asbestos workers were informed about the study, asked to sign the consent form and complete the questionnaires to obtain necessary information about their life style and personal habits (age, working duration smoking habits, health etc.) elaborated to determine the profile and habits of study population. The protocol has been approved by local ethical committee. The exposed workers to asbestos the duration of service was taken more than five years.

B. PREPARATION OF BUCCAL CELL SAMPLING

Prior to buccal cell collection the asbestos 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 buccal samples were transferred to eppendorf tubes with PBS (Phosphate Buffer saline) solution at P^H . 7.0 and centrifused 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 were then air dried and stained with 2% Giemsa for 10 Min. The slides were observed under microscope for the presence of micronuclei. Scoring criteria for buccal cytome assay is three

slides were scored for each sample. 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.

C. SCORING METHOD AND STATISTICAL ANALYSIS

To determine the frequency of various cell types, about 1000 cells were screened for the presence of micronucleated, binucleated, karyohectic and karyolytic cells. All the data were expressed as the Mean Standard Deviation. The synergistic effect between control 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 t- test.

D. RESULTS AND DISCUSSION

Table 1 shows the main characteristics in control case studies. The mean age group of the selected workers belongs to the range from 43.33 ± 7.3 in control group and from 43.52 ± 6.8 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 reveals micronuclei and binucleated cells of buccal smears. The frequency of micronuclei (fig:2), binucleated cells (fig :3), karyohectic cells and karyolysis cells (fig :4) in Asbestos industry workers were shown in table 2. The mean value of micronuclei in smokers was 9.20 ± 0.14 as against 6.40 ± 0.80 in non smoker exposed group. The mean value of binucleated cells in subjects without smoking was 5.40 ± 1.08 as against 6.80 ± 1.01 in subjects with a habit of smoking. The mean value of karyohectic cells (KRC) in smokers was 15.12 ± 0.80 as against 10.20 ± 0.68 in non smoker exposed group. The mean value of karyolytic cells (KLC) cells in subjects without smoking was 32.12 ± 0.10 as against 46.80 ± 0.60 in subjects with a habit of smoking. The values were significantly higher in smokers of exposed subjects compared to non smokers of exposed group. Thus indicating that habit of smoking enhanced the mean values of KRC and KLC nuclear anomalies when compared to control values. The frequency of micronucleate cells, binucleate cells, Karyohectic and Karyolysis cells were compared in duration of exposure less than 5 years and in ten years exposure and it is more significantly higher in ten years of exposed (Maluf 2000).

Group	No. of subjects	Age in years	Duration of exposure
Control	88	43.33 ± 7.3	19.5 ± 2.8
Exposed	108	43.52 ± 6.8	19.8 ± 2.91
Non smokers	68	42.11 ± 8.01	19.5 ± 2.27
Smokers	40	42.83 ± 7.01	19.3 ± 2.64

Table 1: Demographic characteristics of study subjects

Individuals	MNC	BNC	KRC	KLC
Control	3.62 ± 0.22	4.60 ± 0.12	11.30 ± 0.16	32.10 ± 0.26
Smokers (N=30)	2.18 ± 0.18	3.0 ± 0.12	9.10 ± 0.96	18.60 ± 1.06
Non Smokers (N=36)				
Exposed	$9.20 \pm 0.14^*$	$6.80 \pm$	$15.12 \pm$	$46.80 \pm$

Smokers (N=30)	6.40 ± 0.80	1.01* 5.40 ± 1.08	0.80* 10.20 ± 0.68	0.60* 32.12 ± 0.10
Non Smokers(N=36)				
Duration of exposure	4.80 ± 0.42	5.40 ± 0.80	18.00 ± 1.02	30.60 ± 1.06
5 Years (N=40)	10.20 ± 0.82	10.60 ± 0.92	21.80 ± 0.80	44.20 ± 0.94
10 Years (N=48)				

*P<0.05

Table 2: Frequency of micronuclei in Asbestos industry workers

Micronucleus test of exfoliated cells in epithelial tissue have been used to evaluate the genotoxic effects. Micronucleus is defined as microscopically visible, round or oval cytoplasmic chromatin mass next to the nucleus. Micronuclei originated from aberrant mitosis and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated in to the daughter nuclei during mitosis. Micronucleus test is the most frequent technique used to detect chromosome breakage or mitotic interference associated events with increased risk for cancer (Curtis 1982). As micronuclei derive from chromosomal fragment and whole chromosomes lagging behind in anaphase, the micronucleus assay can be used to show both clastogenic and aneugenic effects. Micronucleus formation is undoubtedly an important mechanism for chromosome loss (Ford1992).

Continuous efforts have been made to identify genotoxic agents, to determine conditions of harmful exposition and to monitor populations that are affected. The main objective of the study was to evaluate if the exposure to complex mixture of chemicals in construction, induced increase in the level of genetic damage. The study was carried out in parallel with exposed and control group both from the same area and with similar individual characteristics. To evaluate the genetic damage one of the most common biomonitoring methods Micronucleus assay was chosen. Biomarkers also permit enhanced analysis of health risk in humans exposed to carcinogens and because determinations are performed directly in human organism, uncertainties inherent in epidemiologic studies are avoided. There is no study available on the biomonitoring of construction workers. The current investigation reports present investigation recommended that construction workers under their particular conditions of exposure (tobacco and alcohol) reveal clear evidence of genotoxicity in buccal cells when evaluated by MN test.

Our study revealed a significant induction of micronuclei in construction workers when compared to controls with respect to their age, years of exposure, smoking etc..Similarly, an increased incidence of chromosomal aberrations was observed in the cement factory workers with smoking habit (Shehla et al., 2001). These results are in agreement with our previous study which reported an increased chromosomal aberration among cement factory workers (Jude et al., 2002) and an increased MN induction in cement exposed tobacco chewers (Sudha et al., 2009). An enhanced sister chromatid exchange and chromosomal aberration in peripheral blood lymphocytes of asbestos factory workers were reported by Fathima et al (2001) and also cement particulate extracts in invitro showed an increased chromosomal aberration (Hadnag et al., 1989).

The micronuclei in exfoliated epithelial cells are useful biomarkers of occupational exposure to genotoxic chemicals. Cigarette smoking is one of the factor that may influence the rate of DNA damage such as incidence of micronuclei in humans (Celik et. al. 2003) reported that cigarette smoking significantly increase the frequencies of nuclear abnormalities in both controls and exposed subjects. Increase in exposure to toxic chemicals such as formaldehyde and benzeze induces a significant increase in the buccal cell micronuclei (Titenko Holland et. al. 1996, Suralles et al. 1997) copper smelters (Levinenska et. al 2007), shoe factory workers (Gian et. al. 2009).

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

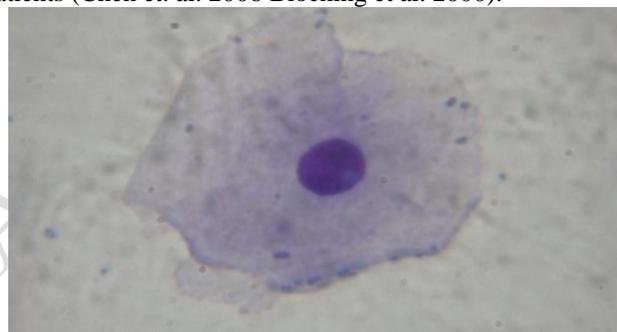


Figure 1: Normal Buccal cell

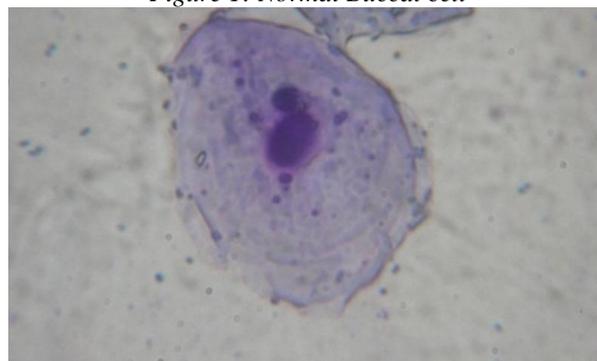


Figure 2: Micronucleated buccal cell



Figure 3: Binucleated Buccal cell



Figure 4: Karyolytic Buccal cell

III. CONCLUSION

Our findings conclude that asbestos exposure causes instability of the genetic material in the workers and can be taken as an indication that these individuals have increased cancer risks. To enable a better assessment of the relative importance of dermal versus inhalation exposure, further quantitative data on uptake of asbestos dust via the skin would be needed. Quantitative data on dermal uptake of asbestos among exposed workers, relative to the inhalatory dose will enable a health risk assessment. This would require well-designed field studies with small groups of exposed workers either (i) solely skin exposed or (ii) solely with inhalation and (iii) a group with both dermal and inhalatory exposure cautiously. In conclusion, biomonitoring studies of workers exposed to construction industry are rather vague because each population has a different life style factors but same occupation in different areas under different climatic and environmental conditions and are exposed to indistinguishable mutagen. Therefore, there is a need to educate those who work in construction sites about the potential hazard of occupational exposure and the importance of using protective measures. Since DNA damage is an important step in events leading from carcinogen exposure to cancer disease.

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REFERENCES

[1] A.N.M.Abou Taleb, A.O.Musaniger, R.B.Abdel Moneim; J.Roy. (1995).*Soc.Health*, 2, 378-383
 [2] Abu Dhaise BA, Rabi AZ, al Zwairy MA, et al (1997). Pulmonary manifestations in cement workers in Jordan. *Int J Occup Med Environ Hlth*, 10, 417-28
 [3] Algranti E, Mendonca EM, DeCapitani EM, et al (2001). Nonmalignant asbestos- related diseases in Brazilian asbestos cement workers. *Am J Ind Med*, 40, 240-54.
 [4] Al-Neaimi YI, Gomes J, Lloyd OL (2001). Respiratory illnesses and ventilatory function among workers at a

cement factory in a rapidly developing country. *Occup Med (Lond)*, 51, 367-
 [5] Bloching M, Hofmann A, Lautenschlager C, Berghaus A and Grummt T (2000) Exfoliative Cytology buccal mucosa to predict the relative risk of cancer in the upper aerodigestive tract using the MN *Oncol* 36 : 550-555.
 [6] Celik A, Cava T and ErgeneGozukara S (2003) Cytogenetic Biomonitoring in petrol station attention Micronucleus test in exfoliated buccal cells. *Mutagenesis* 18: 417421.
 [7] Celik A, Kanik (2006) "A Genotoxicity of Occupational Exposure to wood dust: Micronucleus frequency nuclear changes in exfoliated buccal cells". *Environ Mol Mutagen* 47 (9) 693 – 698.
 [8] Champlon M and Tahnuy (1977): Asbestos and glass fibres in bacterial mutation test *Mut. Res* 43:159-164.
 [9] Chen C, Arjomandi A, Qin H, Balmes J, Tager I and Holland N (2006) Cytogenetic damage in buccal and peripheral lymphocytes of young healthy individuals exposed to ozone. *Mutagenesis* 21:131.
 [10] Dedobbeler N, F.Baland; (1991) *Journal of Safety Research*, 22(2), 97-103
 [11] Dong F, Deng J, Pu ZX and John H (2006) Pulmonary alveolar macrophage cytotoxicity investigation shape mineral dusts. *Sheng Wu Yi Xue Gong Cheng XueZaZhi* 23 848 51.
 [12] Fatima SK, Prabhavathi PA, Padmavathi P, et al (2001). Analysis of chromosomal aberrations in men occupationally exposed to cement dust. *Mut Res*, 490, 179-86
 [13] Fenech M, (2007). "Cytokinesis-block micronucleus cyto assay", *Nat. Protoc.*, vol. 2, pp. 1084-1104.
 [14] Fenech M, Bolognesi C, KrischVolders M, Bonassi S, Zeiger E, Knasmuller S and `Holland N (2001) Harmonisation of the micronucleus assay in human buccal cells A Human Micronucleus (HUMN) initiative commencing in 2007. *Mutagenesis* 22:3-4.
 [15] Fenech M., (1993) "The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations", *Mutat. Res.*, vol. 285, pp. 35-44.
 [16] Guthrie Jr GD (1992) Biological effects of inhaled minerals. *Am Mineral* 77: 225 243.
 [17] Hadnagy W, Seemayer NH, Tomingas R, et al (1989). Comparative study of sister chromatid exchanges and chromosomal aberrations induced by airborne particulates from an urban and highly industrialized location in human lymphocyte cultures. *Mut Res*, 225, 27-32
 [18] J.H.Ford, A.T.Correll; (1992) *Genome.*, 35, 702-705
 [19] J.R.Curtis, B.B.Parida; (1982) *Int.J.Cancer*, 30, 553-559
 [20] Jakobsson K, Attewell R, et al (1990). Mortality and cancer morbidity in cohorts of asbestos cement workers and referents. *Br J Ind Med*, 47, 602-10.
 [21] Jakobsson K, Horstmann V, Welinder H (1993). Mortality and cancer morbidity among cement workers. *Br J Ind Med*, 50, 264-72
 [22] Jarvholm B (2006). Carcinogens in the construction industry. *Ann N Y Acad Sci*, 1076, 421-8
 [23] Jaurand MC (1997): Mechanisms of fibre induced genotoxicity *Env. Health percep*:105: 1073-1084

- [24] Jude, C.A.L., Sasikala, K., Ashok Kumar, R., Sudha, S., Raichel, J. (2002). Haematological and cytogenetic studies in workers occupationally exposed to cement dust. *Int. J. Hum. Genet.* 2(2): 95-99.
- [25] Kalyanaswamy B, Kameshwari and Rudrama Devi K. (1993): Chrysotile asbestos induced mesonucleic in bone marrow erythrocytes of swiss albino mice. *Inter. Agri. Biol. Re* 9(2) 46-49
- [26] Lavappa K., S. F4 and S.S. Epstern (1974): Cytogenic studies on chrysotile asbestos *Env. Res.* 10:165-173.
- [27] Laxman Rao P, Ravi M, Rupa D S and P.P Reddy (1987): Effect of chrysotile asbestos on bone marrow cells *Proc. Int. Gen.* 99-103.
- [28] Levinenska AJ, Savian A, Talaksa G, Boeniger MF, Suruda A and Schulte PA (1997), the Utility of epit Micronuclei in the assessment of intermittent exposure. *Biomarkers* 2.
- [29] Maciejewska, G. Bielichowska-Cybula; (1991). *Med.Pr.*, 42(4), 281-290.
- [30] Manning CB, Vallyathan V, Mosmam BT (2002): Disease caused by asbestos: Mechanisms of injury and disease development. *Int. Immuno. Pharmacology*: 21: 191-200.
- [31] Mossmar BT, Gee JB, (1989): Asbestos related disease, *N Eng J Med*, 320: 1721-17
- [32] Olsen J, Sabroe S (1984). Occupational causes of laryngeal cancer. *J Epidemiol Community Hlth*, 23, 117-21
- [33] Pastor S, Creus A, Xamena N, Siffel C and Marcos R (2002). Occupational exposure to pesticides damage: Results of a Hungarian population study using the micronucleus assay in lymphocytes, *EnvrionMol Mutagen* 40: 101 -109.
- [34] Prioia NK, Paszkiewicz GM, Nasca MSS, Franke GE and Paul JL (2006) Smoking and smokeless tobacco associate human buccal cell mutations and their association with oral cancer A review. *Cancer Biomarkers Prevent* 15: 1061 1077.
- [35] Reiss B., S. Soloman C Tong, M Leven stein, S. H Rosenberg G M Williams (1982): Absence of mutagenic acivity of three forms of asbestos in liver epithelial cells. *En. Rs.* 27; 389-397.
- [36] S.W.Maluf, B.Erdtmann; (2000). *Genet.Mol.Biol.*, 23, 485488
- [37] Shehla, K. F., Prabhavathi, P. A., Padmavathi, P., & Reddy, P. P. (2001). Analysis of chromosomal aberrations in men occupationally exposed to cement dust. *Mutation Research*, 490: 179-186.
- [38] Stern F, Lehman E, Ruder A (2001). Mortality among unionized construction plasterers and cement masons. *Am J Ind Med*, 39, 373-88.
- [39] Surralle's J. Carbonell E, Marcos R, Derassi F, Antoccia A and Tanzarella C, (1992). A case study on the improvement of micronucleus test in cultured human lymphocytes, *Mutagenesis*, 7: 407-4010.
- [40] Titenko – Holland N, Moore LE, Smith MT, (1994) Measurement and characterization of micronuclei in exfoliated human cells by uorescence in Situ hybridization with a centrometric probe. *Mut Res.* 313:39-50
- [41] Tolbert PE, Shy CM and Allen JW (1992) Micronuclei and other nuclear anomalies in buccal smears: Methods development. *Mutat Res* 271:69-77
- [42] Yang CY, Huang CC, Chiu HF, et al (1996). Effects of occupational dust exposure on the respiratory health of Portland cement workers. *J Toxicol Environ Hlth*, 49, 581-8