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Occupational Exposure as a Risk Factor to Enhance the Risk of Early Ageing

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ABSTRACT

Genotoxic effects of hazards at the environmental and occupational exposure contribute to ageing acceleration. These compounds induce genetic damages in the exposed cells such as buccal cells. The study was carried out on the mechanical workshop workers to investigate micronuclei (MN) formation in the exposed subjects. The formation in the cells was evaluated by using MN test. The applied buccal cells as sensitive sources showed the genotoxic and cytotoxic damages which could accompany ageing. Difference in MN frequency between the workers and controls was statistically significant ($p = 0.001$). It showed statistically significant difference between the older and younger groups in the workers and controls ($p = 0.001$) as well. The results showed that the occupational exposure increased MN frequency in the workers. It was concluded that the induced genomic damages could act as an accelerating factor to enhance the speed of ageing in the workers.

Key words: MN test, ageing, occupational exposure, buccal cells, cytogenetic damage

INTRODUCTION

The accumulated mutations in the genome of somatic cells could result in ageing and its consequences. It leads to tissue atrophy, development of neoplastic process and reduced functions of the organs and tissues in the body (Bhattacharya, 2011; Vijg, 2000). Health is influenced by different factors such as inherited, nutritional and environmental factors. People living in the industrialized areas are intensely exposed to the chemical substances, which potentially could be mutagenic (Martino-Roth *et al*., 2002). Ageing appears to be due to combination effects of both genetic and environmental factors, which occurs at the cellular level (Saeed *et al*., 2005; Wojda and Witt, 2003). The mechanical workshop workers are exposed to compounds of different agents such as petroleum. The workers are occupationally exposed to the chemicals during repairing the cars and motorbikes in the workshops (Celik *et al*., 2003). Occupationally exposure to the mixture of chemical compounds with genotoxic effects in the workshops predisposes workers to the genetic material damage (Pitarque *et al*., 1997). Acquired data from the hazards effects studies help to protect the individuals against the potential harm of exposure, such as cancer and other effects of early ageing (Celik *et al*., 2003).

The exfoliated buccal mucosa cells are strong potential sources in human population for biomonitoring studies in the exposed individuals. The cells could be easily collected from the mouth

by a non-invasive procedure (Torres-Bugarin *et al.*, 2004). The cells are excellent sources for monitoring the exposure to the occupational and environmental hazards due to the direct route of exposure to pollutants. In addition, buccal cells are capable to metabolize the carcinogen compounds to reactive chemicals (Salama *et al.*, 1999). Since, the cells express the genotoxic effects, they are used to reveal the occupational exposure and compounds effects on MN formation (Heuser *et al.*, 2007).

Micronucleus arises from acentric fragments or whole chromosomes in the anaphase period, which is not localized in the main nuclei of the daughter cells (Jiraungkoorskul *et al.*, 2007a; Martins *et al.*, 2009). MN is a reliable biomarker, which shows the ability of agents to cause chromosome loss (Alarifi *et al.*, 2009; El-Shahaby *et al.*, 2003; Jiraungkoorskul *et al.*, 2007b; Nirmala *et al.*, 2008; Qurtam *et al.*, 2009; Sellappa *et al.*, 2009). It is not repairable and stands as evidence that genetic damages had occurred in dividing basal cells, one or three weeks earlier (Ray *et al.*, 2005). It could be used as a sensitive cytogenetic biomarker and rapid indicator of genetic damages in the exfoliated cells as a result of the occupational exposure (Wu *et al.*, 2004). MN test shows the frequency of MN biomarker, which evaluates the risks of environmental exposure. It presents the susceptibility status of taking harm in the exposed population (Balakumar *et al.*, 2010; Martins *et al.*, 2009; Qurtam *et al.*, 2009; Wu *et al.*, 2004). The test in the cells reflects the genotoxic effects of hazards that might occur. It could be used directly in the buccal cells without the requirement for the cell culture or metaphase preparation (Torres-Bugarin *et al.*, 2004).

In the current study, MN frequency was evaluated in the epithelial buccal cells collected from the workers and controls to monitor the induced cytotoxic effects of hazards in the exposed cells, which influence ageing (Martins *et al.*, 2009). This study aimed to identify the increased MN frequency at the occupational exposure in relation to the speed of ageing through the induced cytotoxic damages in the exposed individuals.

MATERIALS AND METHODS

The study was carried out in Medicine and Health Sciences Faculty of Universiti Putra Malaysia (UPM) in 2010. Approval and permission of study were obtained from the ethical committee in the faculty (Reference Number: UPM/FPSK/PADS/T7-MJKEtikaPer/F01 (JSB-Aug (08)05). In the current study, 120 car mechanical workshop workers were considered as cases that were occupationally exposed. The same number of individuals was used as control group, without a history of the occupational exposure in the workshops.

The exposed individuals were comprised of males, who worked in the workshops at least one year. Subjects were interviewed about their work history and duration of working time, state of health, smoking habit and other aspects relevant to the study. The exfoliated cells of buccal mucosa were obtained by scraping with a cytology brush in the oral cavity. The mouth rinsed with water before the sampling begins. The cells were collected by scraping the inner part of both sides of the cheeks three times with cytology brush. Then, the cells were gently mixed with 0.9% sodium chloride in a 1.5 mL eppendorf tube. The cells were centrifuged for 1 min at 2500 rpm. The supernatant was removed and cells were smeared onto two clean slides by pulling method.

The cells were allowed to air dry before staining procedure took place. The epithelial cells smeared on the slides, dried and fixed with cold solution of 1% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.5) for 20 min. Then the slides were stained by feulgen reaction using the modified procedure of Belien *et al.* (1995). Firstly, the cells were hydrolyzed in 5 N HCl at 27°C for

30 min. Then the slides were washed in distilled water for five min and stained with fresh Schiff reagent (Sigma Chem, USA) for 45 min. Lastly, the slides were washed by tap water for 15 min and then counter stained with 0.1% naphtol-yellow (Sigma-Aldrich, India) for 20 sec. For analysis 2000 cells of each sample were counted and analyzed under light microscope with 200X magnification dry lens objective. Results expressed as frequencies of the micronucleated epithelial cells in the 2000 cells.

Statistical analysis: The normality of variables was evaluated by the Kolmogorov-Smirnov test (Rohr *et al*., 2006). The Mann-Whitney U-test and ANOVA were used to compare the groups. The statistical analysis of differences in MN frequency in the groups was carried out using the non-parametric Mann-Whitney U-test. Correlations between the different variables were determined by Spearman rank correlation test. The critical level for rejection of the null hypothesis was considered to be a p value of 5%. All analyses were performed using the statistical package for the social sciences (SPSS) (Chicago, IL) software version 16.0.

RESULTS

In this study, MN formation represented as a character of genetic material damage in the buccal cell samples (Fig. 1). Results showed a statistical significant difference in MN frequency between the workers and controls ($p = 0.001$). The minimum of MN frequency in the workers and controls was 5.33 and 0.00, respectively. The maximum of MN frequency in the workers was 20.67 that it was two-fold higher than the controls (2.14 times). The mean MN formation was 12.29±4.34 in the workers and 2.44±1.82 in the controls. MN differentiated significantly between the workers and controls ($p<0.05$) (Table 1). The individuals were divided into two groups of older (≥30)

Table 1: Summary and comparison of MN frequency between the workers and controls

Study group	N	Mean±SD	Min.	Max.	p-value
Workers	120	12.29±4.34	5.33	20.67	0.001[a]
Controls	120	2.44±1.82	0.00	9.67	

[a]Significant at the 0.05 level using two-tailed students t-test

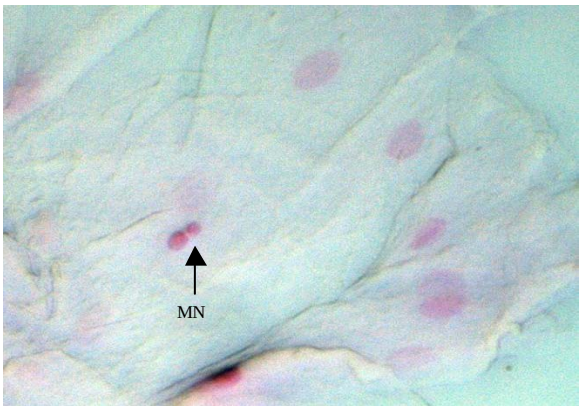

Fig. 1: Buccal epithelial cell, representative micronuclei (MN) for Micronuclei test observed in the cell (arrow) as compared to absent MN in neighbor cells (Magnification 200X)

Table 2: Correlation between age and MN frequency in the individuals

Study groups	Test	r	p-value
Workers Controls	Spearman	0.690	0.001
Workers		0.707	0.001
Controls		0.703	0.001

Correlation is significant at the 0.01 level (2-tailed) by spearman test

Table 3: Results of age effects on MN frequency level among individuals

Tail length/age	N	≥30 Mean±SD	N	<30 Mean±SD	p-value
All subjects	65	14.06±4.83[a]	175	4.88±4.14[b]	0.001**
Workers	54	15.60±3.59[c]	66	9.59±2.72[d]	0.001**
Controls	11	6.45±2.03[e]	109	2.04±1.21[f]	0.001**
p-value		0.001*		0.001*	

Means with different superscripts are significant at p<0.05 using the Mann-Whitney U-test, *p-value is between the workers and controls in each column. **p-value is between age groups (<30≤) in each row

Table 4: Result of MN biomarker changes with socio-demographic factors in the workers and controls

	Workers		Controls		All subjects		
Group	N	Mean±SD	N	Mean±SD	N	Mean±SD	p-value
All subjects	120	13.19±0.77[a]	120	3.11±0.96[b]	240	8.15±0.60	0.001
Smokers	59	13.69±1.05	30	3.21±1.30	89	8.45±0.90	0.494
Non-smokers	61	12.70±0.89	90	3.01±1.02	151	7.85±0.72	
Educated	24	13.59±1.31	105	3.41±0.75	129	8.50±0.86	0.590
Non-educated	96	12.80±0.55	15	2.81±1.59	111	7.80±0.91	
Drinkers	12	13.51±1.13	3	3.42±1.25	3	8.47±1.00	0.520
Non-drinkers	108	12.88±0.65	117	2.79±0.90	117	7.83±0.46	
Malay	65	11.85±1.03	78	3.32±1.02	143	7.58±0.97[a]	0.004
Chinese	45	13.59±0.79	33	2.30±1.08	78	7.95±0.72[a]	
Indian	10	14.15±1.94	9	3.70±2.24	19	8.92±1.20[b]	
Working time>5Y	37	14.38±0.93[a]		-			0.001
Working time<5Y	83	11.18±0.79[b]		-			

Means with different superscripts are significant at p<0.05

and younger (<30). MN formation showed significant and direct correlation with age in the workers (r = 0.707, p = 0.001), controls (r = 0.703, p = 0.001) and all individuals (r = 0.690, p = 0.001) (Table 2). It indicated that MN frequency increased with age. Statistically significant difference in MN formation was observed between the workers and controls in the older and younger groups (p = 0.001). Meanwhile, MN formation difference between the older and younger groups was significant (p = 0.001) in the workers and controls (Table 3).

Difference in MN frequency was significant between the workers and controls (p = 0.001). The socio-demographic factors of smoking, educational level and alcohol consumption did not show any statistically significant effects (p>0.05) on MN formation in the workers as compared to the controls. Working time expressed significant difference (p = 0.001) in MN formation between the workers and controls. Ethnicity showed statistically significant effect on MN between the workers and controls among Chinese, Malays and Indians (p = 0.004) (Table 4).

DISCUSSION

MN frequency was 12.29±4.34 in the car mechanics, which was about five fold higher than the controls with MN frequency of 2.44±1.82. It revealed that the mechanics had a higher frequency of MN, as compared to the controls. The association of MN frequency with age in the individuals showed a direct and significant correlation ($r = 0.690$, $p = 0.001$). The average MN frequency in the healthy population is about one to three per 2000 cells, regardless of different types of cells (Speit and Schmid, 2006). Maximum presented MN frequency in the car mechanics and controls was 20.67 and 9.67, respectively.

MN formation in the workers was approximately 2 fold higher than the controls. Statistical significant difference in MN frequency between the workers and controls confirmed the findings from the previous studies, which reported the increased MN formation in buccal cells among car mechanics, painters and battery renovation workers as compared to the controls (Benites *et al.*, 2006; Hallare *et al.*, 2009; Martino-Roth *et al.*, 2002, 2003).

All subjects in this study were divided into two groups of older and younger within the intervals of <30 or ≥30. The statistical analysis showed significant difference ($p<0.05$) in MN frequency between age groups in all individuals, the workers and controls. Difference in MN formation was significant ($p<0.05$) as well in the older and younger groups between the workers and controls (Table 3). This study confirmed the previous reports about the occupational exposure effects on the increased MN frequency and ageing (Benites *et al.*, 2006). Since, MN is a valid biomarker of ageing (Wojda and Witt, 2003), genetic damages caused by the occupational exposure accelerate ageing and consequences (Thomas, 1995). Although, some researchers reported no significant correlation between ageing and MN frequency in the exposed cells (Cavallo *et al.*, 2007; Sambyal *et al.*, 2004).

Hence, significant association between age and MN frequency in this study indicated that this biomarker accompanied growing up and being old. Presence of MN along with ageing caused by DNA damage results synergic or additive effects to accelerate ageing. Different studies tried to identify the higher risk groups, who need more intervention and control (Mooney *et al.*, 1997; Norppa, 2004). The results of studies could be influenced by lifestyle factors such as smoking and smoking intensity, nutrition and knowledge (Ada *et al.*, 2007). In this study, the socio-demographic factors of smoking, alcohol consumption, educational level and duration of working time were studied. Statistically significant difference in MN frequency between the workers and controls among Malays, Indians and Chinese showed ethnicity effect as a reason of inter-individual variations (Hewakapuge *et al.*, 2008).

Duration of working time affected significantly ($p = 0.001$) the biomarker in the individuals (Table 4). It implied possibility of higher genomic damage in relation to duration of exposure time (Manikantan *et al.*, 2010, 2009; Pinto *et al.*, 2000). Statistically non-significant effect of educational level on MN frequency showed that knowledge by improving lifestyle and healthy life could prevent genomic damages and MN formation (Pavanello *et al.*, 2005). Overall, the results could be influenced by different factors of various ethnicity, sample size, type of tissue, present conditions in laboratory and many other differences present in the studies (Hewakapuge *et al.*, 2008).

CONCLUSION

The results revealed that the occupational exposure as an environmental exposure could increase genetic material damage in the exposed people, which accelerate ageing. The results of study indicated that the mechanical workshop workers experienced the genotoxic exposure, which was reflected by in a high level of MN frequency. Although the responsible factors for the reported

genetic damages were not identified, the present study indicated that these damages could accompany ageing in the workers. The findings are helpful to identify the susceptible individuals for effective intervention through a suitable lifestyle. For taking a good prediction about the effect of this biomarker, it is needed to choose suitable sample size in each group of age.

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REFERENCES

Ada, A.O., M. Yilmazer, S. Suzen, C. Demiroglu and A.E. Demirbag *et al.*, 2007. Cytochrome P450 (CYP) and glutathione S-transferases (GST) polymorphisms (CYP1A1, CYP1B1, GSTM1, GSTP1 and GSTT1) and urinary levels of 1-hydroxypyrene in Turkish coke oven workers. Genet. Mol. Biol., 30: 511-519.

Alarifi, S.A. S. Alkahtani, F.M.A. Tarboush and A. Al-Qahtani, 2009. Effect of DNA hypomethylation on genotoxicity and apoptogenicity of sodium arsenite in laboratory mice. Pak. J. Biol. Sci., 12: 554-564.

Balakumar, B.S., R. Suresh and R. Venugopal, 2010. Modulatory effects of ascorbic acid and α-tocopherol on arsenic induced micronuclei formation. Int. J. Pharmacol., 6: 676-680.

Belien, J.A.M., M.P. Copper, B.J.M. Braakhuis, G.B. Snow and J.P.A. Baak, 1995. Standardization of counting micronuclei: Definition of a protocol to measure genotoxic damage in human exfoliated cells. Carcinogenesis, 16: 2395-2400.

Benites, C.I., L.L. Amado, R.A.P. Vianna and M.D.G. Martino-Roth, 2006. Micronucleus test on gas station attendants. Genet. Mol. Res., 5: 45-54.

Bhattacharya, S., 2011. Natural antimutagens: A review. Res. J. Med. Plant, 5: 116-126.

Cavallo, D., C.L. Ursini, E. Omodeo-Sale and S. Iavicoli, 2007. Micronucleus induction and FISH analysis in buccal cells and lymphocytes of nurses administering antineoplastic drugs. Mutat. Res., 628: 11-18.

Celik, A., T. Cavas and S. Ergene-Gozukara, 2003. Cytogenetic biomonitoring in petrol station attendants: Micronucleus test in exfoliated buccal cells. Mutagenesis, 18: 417-421.

El-Shahaby, O.A., H.M.A. Migid, M.I. Soliman and I.A. Mashaly, 2003. Genotoxicity screening of industrial wastewater using the *Allium cepa* chromosome aberration assay. Pak. J. Biol. Sci., 6: 23-28.

Hallare, A.V., M.K.R. Gervasio, P.L.G. Gervasio and P.J.B. Acacio-Claro, 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.

Heuser, V.D., B. Erdtmann, K. Kvitko, P. Rohr and J. da Silva, 2007. Evaluation of genetic damage in Brazilian footwear-workers: Biomarkers of exposure, effect and susceptibility. Toxicology, 232: 235-247.

Hewakapuge, S., R.A.H. van Oorschot, P. Lewandowski and S. Baindur-Hudson, 2008. Investigation of telomere lengths measurement by quantitative real-time PCR to predict age. Legal Med., 10: 236-242.

Jiraungkoorskul, W., P. Kosai, S. Sahaphong, P. Kirtputra, J. Chawlab and S. Charucharoen, 2007a. Evaluation of micronucleus test`s sensitivity in freshwater fish species. Res. J. Environ. Sci., 1: 56-63.

Jiraungkoorskul, W., S. Sahaphong, P. Kosai and M.H. Kim, 2007b. Micronucleus test: The effect of ascorbic acid on cadmium exposure in fish (*Puntius altus*). Res. J. Environ. Toxicol., 1: 27-36.

Manikantan, P., V. Balachandar, K. Sasikala and S. Mohanadevi, 2009. DNA damage in viscose factory workers occupationally exposed to carbon di-sulfide using buccal cell comet assay. Braz. J. Oral Sci., 8: 197-200.

Manikantan, P., V. Balachandar and K. Sasikala, 2010. DNA damage in workers occupationally exposed to lead, using comet assay. Int. J. Biol., 2: 103-110.

Martino-Roth, M.G., J. Viegas, M. Amaral, L. Oliveira, F.L.S. Ferreira and B. Erdtmann, 2002. Evaluation of genotoxicity through micronuclei test in workers of car and battery repair garages. Genet. Mol. Biol., 25: 495-500.

Martino-Roth, M.G., J. Viegas and D.M. Roth, 2003. Occupational genotoxicity risk evaluation through the comet assay and the micronucleus test. Genet. Mol. Res., 2: 410-417.

Martins, R.A., G.A. Gomes, O. Aguiar Jr. and D.A. Ribeiro, 2009. Biomonitoring of oral epithelial cells in petrol station attendants: Comparison between buccal mucosa and lateral border of the tongue. Environ. Int., 35: 1062-1065.

Mooney, L.A., D.A. Bell, R.M. Santella, A.M. Van Bennekum and R. Ottman *et al.*, 1997. Contribution of genetic and nutritional factors to DNA damage in heavy smokers. Carcinogenesis, 18: 503-509.

Nirmala, K., T.P. Krishna and K. Polasa, 2008. Inhibition of induced micronuclei formation in human lymphocytes by ginger. Int. J. Cancer Res., 4: 12-19.

Norppa, H., 2004. Cytogenetic biomarkers and genetic polymorphisms. Toxicol. Lett., 149: 309-334.

Pavanello, S., A. Pulliero, S. Lupi, P. Gregorio and E. Clonfero, 2005. Influence of the genetic polymorphism in the 5'-noncoding region of the CYP1A2 gene on CYP1A2 phenotype and urinary mutagenicity in smokers. Mutat. Res., 587: 56-66.

Pinto, D., J.M. Ceballos, G. Garcia, P. Guzman and L.M. Del Razo *et al.*, 2000. Increased cytogenetic damage in outdoor painters. Mutat. Res.: Genet. Toxicol. Environ. Mutagen., 467: 105-111.

Pitarque, M., E. Carbonell, N. Xamena, A. Creus and R. Marcos, 1997. Genotoxicity of commercial petrol samples in cultured human lymphocytes. Rev. Int. Contam. Ambient., 13: 15-21.

Qurtam, A.A., S. Alkahtani, F.M. Abou Tarboush, S.A. Alarifi, A. Al-Qahtani and M. Al-Eissa, 2009. Effect of the antioxidant butylated hydroxytoluene on the genotoxicity and cytotoxicity induced in mice by sodium arsenite. J. Biol. Sci., 9: 413-422.

Ray, M.R., C. Basu, S. Mukherjee, S. Roychowdhury and T. Lahiri, 2005. Micronucleus frequencies and nuclear anomalies in exfoliated buccal epithelial cells of firefighters. Int. J. Hum. Genet., 5: 45-48.

Rohr, P., J. da Silva, B. Eedtmann, J.A.P. Henriques and K. Kvitko, 2006. The ber pathway genes and pon1 polymorphism: Influence on dna damage in agriculture-exposed workers. Theoria, 15: 69-77.

Saeed, S.A., M.Z.S. Urfy, T.M. Ali, F.W. Khimani and A.U.H. Gilani, 2005. Antioxidants: Their role in health and disease. Int. J. Pharmacol., 1: 226-233.

Salama, S.A., M. Serrana and W.W. Au, 1999. Biomonitoring using accessible human cells for exposure and health risk assessment. Mutat. Res., 436: 99-112.

Sambyal, V., R. Kaur, S. Chaudhary and S. Amar, 2004. High frequency of micronuclei in buccal mucosa of women residing near a sewage disposal drain in Amritsar. Anthropologist, 6: 125-129.

Sellappa, S., M. Balakrishnan, S. Raman and S. Palanisamy, 2009. Induction of micronuclei in buccal mucosa on chewing a mixture of betel leaf, areca nut and tobacco. J. Oral Sci., 51: 289-292.

Speit, S. and O. Schmid, 2006. Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells. Mutat. Res., 613: 1-9.

Thomas, R.D., 1995. Age-specific carcinogenesis: Environmental exposure and susceptibility. Environ. Health Perspect., 103: 45-48.

Torres-Bugarin, O., A. Ventura-Aguilar, A. Zamora-Perez, B.C. Gomez-Meda and M.L. Ramos-Ibarra *et al*., 2004. Evaluation of cisplatin + 5-FU, carboplatin + 5-FU, ifosfamide + epirubicine regimens using the micronuclei test and nuclear abnormalities in the buccal mucosa. Mutat. Res., 565: 91-101.

Vijg, J., 2000. Somatic mutations and aging: A re-evaluation. Mutat. Res., 447: 117-135.

Wojda, A. and M. Witt, 2003. Manifestations of aging at the cytogenetic level. J. Applied Genet., 44: 383-399.

Wu, P.A., C.H. Loh, L.L. Hsieh, T.Y. Liu, C.J. Chen and S.H. Liou, 2004. Clastogenic effect for cigarette smoking but not areca quid chewing as measured by micronuclei in exfoliated buccal mucosal cells. Mutat. Res., 562: 27-38.