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The Association of DNA Damage Level with Early Age at the Occupational Exposure in the Mechanical Workshops Workers

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ABSTRACT

Comet assay is a sensitive, simple and well validated tool for measuring DNA damaged level in single cell. The assay is based on embedding cells in a thin layer of low melting agarose, which is located on the microscopic slide. Comet assay is a method used as an assessment tool for early biological effect biomarker to the occupational exposure. This investigation evaluated the occupational exposure effects in the workers acting as an enhancement factor of ageing. In order to do this assessment 120 workers as exposed and 120 controls as non-exposed were considered. DNA damage was evaluated by measuring the extent of DNA migration in buccal cells. It was found that the mechanical workshop workers who were occupationally exposed to the genotoxic agents for different periods of time recorded significant increased in the levels of DNA damage as compared to the controls. The data were considered for assessment of their correlation with early ageing. The investigation indicated that the responses of DNA damage among the exposed people in the work places could be related to confounding factors such as life styles as well. Comet assay in the evaluation of DNA damage reflected the sensitivity of this method at moment of collecting samples.

Key words: Ageing, occupational exposure, DNA damage, buccal cell, comet assay

INTRODUCTION

Ageing happens at the cellular level under the influence of both combinations of genetic and environmental factors (Eshkoor *et al*., 2011). Damage of nuclear and mitochondrial DNA and diminished DNA repair cause cellular ageing (Wojda and Witt, 2003). In the process, cells exhibit alterations in gene expression, nuclear structure, protein processing and cells metabolism. Morphologically the senescent cells display an increased volume and flattened cytoplasm (Shawi and Autexier, 2008). DNA is a very crucial factor for keeping bodies healthy. DNA is susceptible to damages induced by endogenous and exogenous factors (Wong *et al*., 2005). DNA-repair enzymes repair most damages induced (Jackson and Loeb, 2001), but some escapes repair, causing permanent damages (Wong *et al*., 2005).

The analysis of biomarkers represents the mutagen effects of hazards in the exposed cells. The factors of Reactive Oxygen Species (ROS), chemicals, physical, viruses, life-style factors such as smoking, drinking and nutrition, residential and working areas and seasonal changes can increase the genetic damage levels in buccal cells (Kopjar *et al*., 2006; Nirmala *et al*., 2007). Buccal epithelial cells could be suitable sources for DNA damage evaluation (Eren *et al*., 2002). Direct route of

exposure to ingested pollutants and capability of metabolizing proximate carcinogens to reactive chemicals make these cells as very good sources for monitoring the occupational and environmental exposures (Salama *et al*., 1999).

A sensitive and reliable laboratory tool for investigating of DNA about damage (Griffiths *et al*., 2002; Al-Saleh, 2006), repair (Polasa *et al*., 2006) and protection is needed to determine the potential risk of occupational exposure in the exposed workers (Griffiths *et al*., 2002). The single cell electrophoresis or comet assay is widely used to determine and assess DNA damage level in cells (Griffiths *et al*., 2002; Udroiu, 2007; Da Fonseca *et al*., 2004). It is a rapid, sensitive and inexpensive technique (Kopjar *et al*., 2006; El-Belbasi *et al*., 2008) to evaluate the genotoxic potential of compounds (Ouedraogo *et al*., 2008). The comet in the cells is visualized by DNA staining fluorescent dye and then DNA damage tail length is scored (Wong *et al*., 2005; Gato and Means, 2011). The comet tail length could be a factor to identify the health of people. DNA damage prevention or increased efficiency of repair is beneficial to increase the health in society (Wong *et al*., 2005). Comet assay reflects the current and past exposures to the cells. The actual levels of DNA damage in buccal cells is at the moment of epithelial mucosa sampling (Kopjar *et al*., 2006). Comet assay is used as an assessing tool in DNA damage level at the individual cell level (Burlinson *et al*., 2007). The method detects DNA damage in the cells that occurred prior to the sampling. Therefore, level of DNA damage reveals the true picture of damages that they encountered at that particular time (Kopjar *et al*., 2006).

The present study was undertaken to identify DNA damage level in buccal cells and the association of this variability with external factors such as the occupational exposure. The aim of this study was to determine the effect of the occupational exposure regarding to premature ageing through induced DNA damage in the exposed individuals. Since the mechanics are exposed to many genotoxic substances in the work places, the subjects were considered in the mechanical workshops.

MATERIALS AND METHODS

In this study, the buccal cells were randomly selected from 120 mechanical workshop workers. The sample size was based on the sample size formula. The study design was a cross-sectional study. The investigation was carried out at the Faculty of Medicine and Health Sciences in Universiti Putra Malaysia (UPM). DNA damage level was monitored in the samples by comet assay. Subjects were interviewed about their work history and duration of working time, state of health, smoking and other aspects relevant to the study. The control group included 120 healthy people as volunteers who were not exposed. The age of the workers and controls was 18 years old and above. The exfoliated cells of buccal mucosa were obtained by scraping with a cytology brush in the oral cavity. The mouth was 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 Phosphate Buffered Saline (PBS) in a 1.5 mL Eppendorf tube. The cells were centrifuged for 1 min at 2500 rpm. The supernatant was removed and the cells were kept in the tubes.

Comet assay determined the extent of DNA damage in the cells, which was performed by using the Trevigen CometAssay™ kit protocol (Trevigen, USA). The assay was started immediately after the cells were prepared. Importantly, all work must be done under dimmed light to prevent damage from Ultraviolet (UV) rays. Low Melting-point Agarose (LMA) was melted in a beaker of boiling water, with the cap loosen in 5 min and then was cooled at 37°C water bath for at least 20 min. The cells were combined with LMAgarose at 37°C with ratio 1:10 and immediately 75 μL aliquot was pipetted onto the comet slide in each of the sample area as required. The slides were prepared

in duplicate and placed flat at 4°C in a dark place for 10 min. The slides were then immersed in pre-chilled lysis solution (Trevigen, USA) for 60 min, followed by immersion in freshly prepared alkaline solution, pH>13 for 45 min at room temperature in the dark. After that the slides were placed flat on a gel tray with aligned equidistant from the electrophoresis. The power supply was set at 1 volt per cm (measured from electrode to electrode).

The applied voltage was for 10 min. After that, the slides were stained with 50 μL of diluted SYBR Green (Trevigen, USA) completely before viewing. The slides were examined by using the fluorescent microscope DM 2500 (LEICA, Germany) with magnification 200X and the images were captured. The cells were analyzed by using the commercially available TriTek Comet Score (version 1.5) software (TriTek Corp., Sumerduck, VA, USA). The tail lengths were determined from the center of the head towards the last visible signs at the end of the tail and measured in micrometer.

Statistical analysis: The normality of variables was evaluated by the Kolmogorov-Smirnov test. The Mann-Whitney U-test and ANOVA were used to compare the groups. The statistical analysis of the differences in DNA damage measured by comet assay in the groups was carried out using the non-parametric Mann-Whitney U-test. Correlations between different variables were determined by Spearman rank correlation test. The critical level for rejection of the null hypothesis was the p value of 5% ($p = 0.05$). All analyses were performed using the statistical package for the social sciences (SPSS) (Chicago, IL) software version 16.0.

RESULTS

The comet tail length in the damaged DNA of the cells was a representative of cytotoxic effects of hazards at the occupational and environmental exposure. The damaged DNA showed comet (Fig. 1) in the cells. In our study, the minimum of comet tail length was 5.55 μm in the workers and 1.82 μm in the controls. The maximum of comet tail length was 47.73 μm in the workers that was 1.26 fold greater than the controls (37.91 μm). The mean tail length was 25.13±8.88 in the workers and 17.48±8.00 in the controls. The mean value differed from 17.48±8.00 in the controls with median of 17.00 μm to 25.13±8.88 with median of 23.82 μm in the workers. After measuring the comet tail length in the cells, the mode was used to be analyzed. Comet assay analysis of data

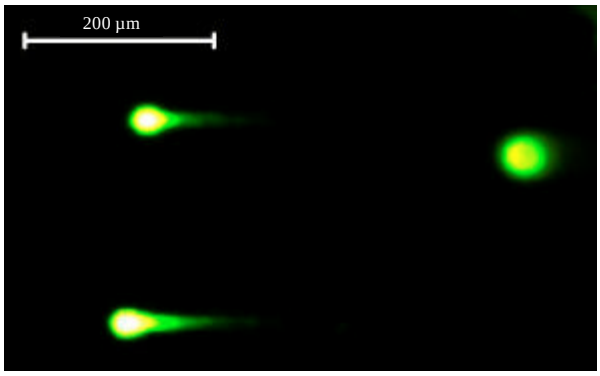

Fig. 1: Buccal cell representative of DNA damage as comet tail length using comet assay

showed that the level of DNA damage differed significantly between the workers and controls ($p = 0.001$) (Table 1). The results of current study revealed that DNA damage level had direct and significant correlation with age in all individuals ($r = 0.601$ $p = 0.001$), the workers ($r = 0.729$ $p = 0.001$) and controls ($r = 0.306$ $p = 0.001$) (Table 2). The people were divided into two groups as older (>30 years) and younger (<30 years). The statistical analysis showed significant difference in comet tail length between age groups in all individuals, the workers and controls ($p<0.05$).

DNA damage tail length difference between the workers and controls was not significant ($p>0.05$) in the older and younger groups (Table 3).

Table 1: Summary and comparison of comet tail length between the workers and controls

Study groups	N	Mean±SD	Min.	Max.	p-value
Workers	120	25.13±8.88	5.55	47.73	0.001[a]
Controls	120	17.48±8.00	1.82	37.91	

[a]Significant at the 0.05 level using the Mann-Whitney U-test

Table 2: Correlation analysis between age and comet tail length

Study groups	Test	r	p-value
All individuals	Spearman	0.601	0.001[a]
Workers		0.729	0.001
Controls		0.306	0.001

[a]Correlation is significant at the 0.01 level (2-tailed)

Table 3: Effect of exposure and age on comet tail length

	Age group ≥30		Age group <30		
Groups	N	Mean±SD	N	Mean±SD	p-value
All individuals	65	20.17±7.69[a]	175	16.98±7.37[b]	0.001**
Workers	54	31.41±7.52[c]	66	19.98±6.21[d]	0.001**
Controls	11	27.91±5.30[e]	109	16.43±7.47[f]	0.001**
p-value	0.900*		0.200*		

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: Comet tail length biomarker changes under influence of the socio-demographic factors in the workers and controls

	Workers		Controls		All subjects		
Groups	N	Mean±SD	N	Mean±SD	N	Mean±SD	p-value
All subjects	120	26.10±2.08[a]	120	19.97±2.59[b]	240	23.03±1.61	0.001
Smokers	59	27.73±2.83	30	22.33±3.48	89	21.04±1.94	0.068
Non-smokers	61	24.47±2.40	90	17.61±2.76	151	25.03±2.43	
Educated	24	26.13±3.52	105	20.80±2.01	129	23.47±2.31	0.959
Non-educated	96	26.07±1.49	15	19.13±4.27	111	22.60±2.44	
Drinkers	12	27.17±3.03	3	21.04±3.35	3	24.11±2.70	0.667
Non-drinkers	108	25.02±1.76	117	18.90±2.41	117	21.96±1.24	
Malay	65	25.33±2.75	78	19.25±2.74	143	22.29±2.59	0.093
Chinese	45	26.11±2.12	33	17.63±2.90	78	21.87±1.93	
Indian	10	26.86±5.20	9	23.03±6.03	19	24.95±3.24	
Working time>5Y	37	32.05±1.80[a]		-			0.001
Working time<5Y	83	21.47±1.53[b]		-			

Means with different superscripts are significant at $p<0.05$

The socio-demographic factors of smoking, educational level and alcohol consumption did not show any statistically significant effect ($p>0.05$) on comet tail length. Duration of working time was divided into more or less than 5 years and showed a significant effect on comet tail length ($p = 0.001$). Ethnicity did not show any statistically significant effect ($p>0.05$) on DNA damage level (Table 4).

DISCUSSION

The occupational exposure may contribute to early ageing and its consequences through mechanisms of genome changes. Several studies evaluated the possible genotoxic effects of the environmental and occupational exposure on the health. Identification of genotoxic conditions helps to determine and monitor populations with excessive exposure (Maluf and Erdtmann, 2000). In comet assay, the comet tail length as a parameter of the cell damage level was used. It was calculated based on the distance from the middle of the comet head to the last visible signal point of the tail (Lima *et al.*, 2008).

Assessment of DNA damage levels in the mechanical workshop workers is applicable by monitoring of occupationally or accidentally exposed to known or potentially genotoxic agents. Different studies showed genetic damage in buccal cells under the influence of various internal and external factors. There are different kinds of risk factors during the life-time of individual that can induce DNA damage in the cells. In this study, the assay was performed on buccal cells from the workers and controls. The tail length of comet was measured in micrometer value. The mean value difference of the two groups showed the greater value of comet tail length in the workers as compared to the controls. For classification of damage, the comet tail length value below 16.67 μm was considered as undamaged, while the greater value was classified as damaged cell (Kopjar *et al.*, 2006).

Based on this classification, the workers presented the cell damages greater than the controls. The result of comet tail length between the workers and controls revealed the potential power of occupational exposure in inducing genotoxicity. It was a confirmation to another study (Cebulska-Wasilewska *et al.*, 2005) expressing statistically significant effect on comet tail length at the occupational exposure. In spite of using the same protocol for all subjects, there were the inter-individual differences to represent DNA damage level. The various levels of damage depended on biological systems and sensitivity of their genomes. Different level of DNA damage in the cells indicated the interaction of induced lesions and repair systems, which demonstrated the level of lesions or high efficiency of repair system (Kopjar *et al.*, 2006).

The results of confounding factors stressed their influences in the increased DNA damage and comet tail length. The results showed a significant association between age and DNA damage tail length. The outcomes of statistical analysis of DNA damage tail length in the younger or older groups confirmed the findings of those who reported no significant effects of exposure in each group of age (Sirota and Kuznetsova, 2008; Kopjar *et al.*, 2006; Zhu *et al.*, 2001; Moller *et al.*, 2000). Although, some studies reported no significant effect of age on DNA damage level (Zhu *et al.*, 2001; Pitarque *et al.*, 1999; Zhu *et al.*, 1999; Palus *et al.*, 1999; Awara *et al.*, 1998; Wojewodzka *et al.*, 1998; Frenzilli *et al.*, 1997; Betti *et al.*, 1994), the results of this study were in a good agreement with those who reported the age-related DNA damage (Maluf *et al.*, 2001; Mendoza-Nunez *et al.*, 2001; Moretti *et al.*, 2000; Piperakis *et al.*, 1998; Singh *et al.*, 1991).

The results demonstrated that ageing tended to increase the level of DNA damage in the vulnerable cells, since represented as the increased comet tail length among the exposed individuals. The limited numbers of subjects in the age groups make it impossible to draw a definite conclusion about association between ageing and DNA damage level. The values of biomarker

indicated that the lifestyle factors and occupational compounds could influence genome damage in buccal cells (Hininger *et al*., 2004; Zhu *et al*., 1999, 2001; Stephan and Pressl, 1999; Palus *et al*., 1999; Piperakis *et al*., 1998; Frenzilli *et al*., 1997; Betti *et al*., 1994).

Some studies reported no significant increase of the biomarker at the exposure (Hoffmann and Speit, 2005; Speit *et al*., 2003; Maluf *et al*., 2001; Pitarque *et al*., 1999; Wojewodzka *et al*., 1999; Sram *et al*., 1998; Kasuba *et al*., 1995; Anderson *et al*., 1988, 1993; Bender *et al*., 1988). It is likely the design of study as well as differences in other lifestyle factors contribute to inconsistent observations. Despite the increased DNA migration in buccal cells of the subjects with the occasional facing of confounding factors, the effects of these infrequent exposures should be carefully evaluated because the number of subjects who reported them was also small for each group. The lifestyle factors, ethnicity and sample size affect the level of DNA damage and ageing at the environmental and occupational exposure.

CONCLUSION

The different studies will help to determine reasons of large differences in ageing among people. In people, ageing would be affected by the environmental and genetic susceptibly factors to induce damaged cells. Occupational exposure is one of the environmental risk factors, threating the human healthy productive life. Despite the limitations, results from the present study revealed the background data that could be used as a value in future genotoxicological monitoring worldwide. It confirmed that comet assay is a sensitive method to detect DNA damage biomarker. It needs further evaluation and standardization for assessing DNA damage in biomonitoring studies as well as in the cases of accidental exposures.

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