GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association of GSTM1 and GSTT1 with ageing in auto repair shop workers

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Genet. Mol. Res. 11 (2): 1486-1496 (2012)
Received June 17, 2011
Accepted February 2, 2012
Published May 21, 2012
DOI http://dx.doi.org/10.4238/2012.May.21.5

ABSTRACT. We evaluated the possible influence of glutathione S-transferase mu (GSTM1) and glutathione S-transferase theta (GSTT1) genes on genetic damage due to occupational exposure, which contributes to accelerate ageing. This study was conducted on 120 car auto repair workshop workers exposed to occupational hazards and 120 controls without this kind of exposure. The null and non-null genotypes of GSTM1 and GSTT1 genes were determined by multiplex PCR. Micronucleus frequency, Comet tail length and relative telomere length differences between the null and non-null genotypes of the GSTM1 gene were significantly greater in the exposed group. Lack of GSTT1 did not affect the damage biomarkers significantly ($P > 0.05$), while lack of GSTM1 was associated with greater susceptibility to genomic damage due to occupational exposure. It was concluded that early ageing is under the influence of these genes and the environmental and socio-demographic factors. Duration of working time was significantly associated with micronucleus frequency, Comet tail length and relative telomere length.

Key words: GST genes; Ageing; Occupational exposure; Comet assay; Micronucleus test; Multiplex PCR

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INTRODUCTION

Senescent cells contribute to ageing and the age-related consequences through several mechanisms (Shawi and Autexier, 2008). Although accumulated cells at the end of the replication period induce ageing (Wojda and Witt, 2003), the mechanism of ageing due to these accumulated cells is still unknown (Shawi and Autexier, 2008). It appears that the loss or altered function of damaged cells induces dysfunction in organs at old age. Ageing is under combination influences of both genetic and environmental factors at the cellular level. Cellular ageing occurs due to nuclear DNA damage, mitochondrial DNA damage and diminished DNA repair (Wojda and Witt, 2003). The glutathione S-transferase (GST) group includes a super-family of proteins, which perform detoxification reactions of xenobiotics (Pande et al., 2008). Enzymes are the most important detoxifying agents in phase II enzymes (Costa et al., 2006), which catalyze mutagenic substrates to inactivate compounds. The GST group includes GSTT1, GSTM1, and GSTP1 genes. Null alleles exist as common forms of GSTT1 and GSTM1 polymorphisms. The total or partial deletion of genes results in non-production of enzymes (Pande et al., 2008).

Many workers in the occupational settings are exposed to different substances. The substances include organic or inorganic chemicals, which are produced or present in the occupational environment in the form of gases, vapors, fumes, mist, or particles. The workers are exposed to complex chemical mixtures in workshops (Benites et al., 2006). Petrochemicals are a group of substances with potential genotoxic effects (Yadav and Seth, 2001). Some researchers studied the effects of genotoxic materials in exposed individuals. Using the related biomarkers helps to identify the damage of DNA or chromosomes at the occupational exposure. The acquired information from the cytogenetic studies could be considered as an early warning to the potential risk of materials, which induce health problems due to longer time of exposure (Martino-Roth et al., 2002). In this study, the mentioned genes encoding the xenobiotic metabolizing enzymes were examined to evaluate their possible influences on the acceleration of ageing by the biomarker alterations of micronucleus (MN), DNA damage tail length and telomere length.

MATERIAL AND METHODS

Approval and permission to perform the study were obtained from the Ethics Committee in Medicine and Health Sciences Faculty (Reference No. UPM/FPSK/PADS/T7-MJKEtikaPer/F01 (JSB-Aug (08)05). The individuals were 120 exposed as workers and 120 non-exposed as controls. The subjects were interviewed to evaluate their health status and lifestyles. The inclusion criteria were considered volunteer car mechanical workshop workers who were above 18 years old without any specific diseases.

The samples were collected from epithelial cells of buccal mucosa. Respondents were asked to rinse their mouth with water before sampling begins. The cells were collected by scraping the inner part of both sides of the cheeks with a cytology brush. Then, the cells were gently mixed with 0.9% sodium chloride and PBS in separate microcentrifuge tubes and brought to the laboratory. The cells were used for MN test, Comet assay and multiplex polymerase chain reaction (PCR). The effects of polymorphisms on the samples were assessed through MN formation,

Comet tail length and relative telomere length as the biological parameters.

Micronucleus test

The cells were smeared on 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 Beliën 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 5 min and after that stained with fresh Schiff reagent (Sigma Chemical, Japan) for 45 min. Lastly, the slides were washed in tap water for 15 min and counterstained with 0.1% naphthol-yellow (Sigma-Aldrich, India) for 20 s. The slides were air dried overnight before viewing the cells under a light microscope. For each sample 2000 cells were analyzed with 200X magnification dry lens objective to obtain MN frequency.

Comet assay

The assay was started immediately after the cells were prepared. Importantly, all work had to be done under dimmed light to prevent cell damage from UV. The cells were combined with LM Agarose at 37°C with a 1:10 ratio and immediately 75 µL of the mixture was pipetted onto the Comet slide for each 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 the pre-chilled lysis solution (Trevigen, USA) for 60 min, followed by immersion in the 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 and aligned equidistant from the electrophoresis. The power supply was set at 1 V per cm (measured from electrode to electrode). The applied voltage was for 10 min. After this step, the slides were stained with 50 µL diluted SYBR Green (Trevigen) completely before viewing. The slides were examined by using the fluorescent microscope DM 2500 (LEICA, Germany) at a 200X magnification 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). Tail lengths of the Comets were determined from the center of the head towards the last visible signs at the end of the tail that was measured on the micrometer.

DNA extraction

Genomic DNA was extracted from the collected samples using the QIAamp DNA blood Mini Kit (Qiagen, Courtaboeuf, France). The purity and concentration of the extracted DNA were measured by the Nanodrop™ 1000 spectrophotometer (Thermo Scientific, USA) and confirmed by resolving DNA on 0.7-1% gel. After preparing the samples to obtain the proper annealing temperature, a gradient PCR was applied in a G-Storm Thermal Cycler (Gene Technology Ltd., UK).

Real-time PCR

Real-time PCR was used to measure the relative telomere length. The primers used

were telomere and 36B4, which were described by Cawthon (2002). The primer sequences were: tel1, GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT; tel2, TCCCGAC TATCCCTATCCCTATCCCTATCCCTATCCCTA; 36B4u, CAGCAAGTGGGAAGGTGTA ATCC and 36B4d, CCCATTCTATCATCAACGGGTACAA. The telomere repeat copy number to single gene copy number (T/S) ratio was determined by Corbett Rotor-Gene 6000 (Corbett Life Science, Sydney, Australia) in 36-well format. For PCR, a 25-µL volume was prepared in the PCR tubes. In each running, both genes telomere and 36B4 were settled for one sample in the separated tubes. The primers were all obtained from Bioline (London, UK). The solution for PCR included 0.6 µL of each primer, 1 µL Eva green, 1 µL DNA, 5 µL master mix Immomix (Bioline), and 16.8 µL pure water. The reaction proceeded 1 cycle at 94°C for 5 min, followed by 40 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 50 s. Melting temperature ranged from 70-95°C. The data from the samples were interpreted throughout the data set to assess the threshold cycle values.

Multiplex PCR

Multiplex PCR technique was applied to determine the presence or absence of GSTT1 and GSTM1 in the samples. The PCR forward and reverse primer sequences for both genes were previously published (Heuser et al., 2007). The primer sequence for GSTM1 were sense: 5'-TTCTGGATTGTAGCAGATCA-3' and anti-sense: 5'-CGCCATCTTGTGCTACATTGCCC G-3' and for GSTT1 were sense: 5'-TTCCTTACTGGTCCTCACATCTC-3', and anti-sense: 5'-TCACCGGATCATGGCCAGCA-3'. The CYP1A1 gene was used as a positive internal control in the reaction based on primers reported by Cha et al. (2007). The primer sequences were sense: 5'-CAGTGAAGAGGTGTAGCCGCT-3' and anti-sense: 5'-TAGGAGTCTTGTCTGATGCCT-3'.

The Multiplex PCR was performed in a 25-µL volume in a 0.2-mL PCR tube. The reaction included 60-100 ng genomic DNA (3-6 µL), 10 pmol of each primer (0.6 µL), 5 µL PCR 5X Master Mix Imomix (Bioline) and 12.8-15.8 µL ddH_2O in a single reaction tube to produce a volume of 25 µL. The PCR was performed under the same conditions within 35 cycles for all samples. The first cycle initiated by incubation at 94°C for 5 min. This was followed by a denaturation stage at 94°C for 30 s, annealing temperature at 62°C arranged based on the gradient PCR applicable for all primers for 30 s, an extension phase at 72°C for 45 s and a final extension at 72°C for 10 min followed the reaction last cycle. After amplification, the samples were stored at 4°C until use. A negative control without DNA template was carried out in each run. PCRs were carried out in the G-Storm Thermal Cycler (Gene Technology Ltd.). The sizes of PCR products were determined by resolving on 1.8-2% agarose gel (Bioline). A 100-bp DNA marker was used as a reference on the gel each time to differentiate the sizes of products. After staining the gel in GelRed Stain (Bioline) for 20 min, it was viewed under UV light by using AlphaImager™ (Alpha Innotech, San Leandro, CA, USA).

Determination of products

Agarose gel electrophoresis running provided the size identification of PCR and RFLP products. The prepared gels for PCR and RFLP products were 2 and 4% respectively. Besides the products, DNA ladders of 50 and 100 bp (Bioline) were also resolved to identify

the product sizes. Finally the gel was viewed under UV light in AlphaImager™ 2200 (Alpha Innotech).

Statistical analysis

The normality of variables was evaluated by the Kolmogorov-Smirnov test. The Mann-Whitney U-test, the independent *t*-test and ANOVA were used to compare the demographic characteristics of study populations. The statistical analysis of differences in the MN test and DNA damage measured by the Comet assay were carried out using the non-parametric Mann-Whitney U-test. Data of telomere length measurement were tested using the independent *t*-test. Gene frequencies were estimated by gene counting, and Hardy-Weinberg equilibrium was evaluated by the chi-squared test for goodness of fit samples. The critical level for rejection of the null hypothesis was considered to be a P value of 5%, two-tailed. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 (Chicago, IL, USA).

RESULTS

Multiplex PCR amplification product was resolved on 2% agarose gel (Figure 1). It determined the presence or absence of GSTT1 and GSTM1 genes in the genomic DNA samples. The results indicated that 62.08 and 26.67% of the individuals were carrying the absence of GSTT1 and GSTM1, respectively (Table 1). The results regarding GSTM1 revealed significant difference in biomarkers of Comet tail length ($P = 0.001$), telomere length ($P = 0.002$) and MN ($P = 0.001$) between the workers and controls in non-null genotype. MN frequency ($P = 0.001$) showed a statistical significant difference between the workers and controls in null genotype. GSTT1 polymorphism showed a statistically significant difference in Comet tail length, telomere length and MN frequency between the workers and controls in all genotypes (Table 2). The effect of these polymorphisms on the biomarkers in the workers or controls was statistically nonsignificant ($P > 0.05$), except a significant effect of GSTM1 on MN frequency in the controls ($P = 0.008$) (Table 3). The effects of polymorphisms on MN frequency, Comet tail length and relative telomere length were assessed in two groups of workers and controls who were younger or older than 30 years of age. The effect of GSTM1 and ageing on MN frequency, Comet tail length and relative telomere length in the workers and controls was not statistically significant ($P > 0.05$). However, it was significant as regards MN formation in the groups of younger ($P = 0.044$) and all ages ($P = 0.008$) in the controls. In GSTT1, the effect of genotypes and ageing on the biomarkers was not statistically significant ($P > 0.05$) (Tables 4 and 5). In this study, ethnicity, working time and lifestyle factors were evaluated. When the socio-demographic factors of smoking habit, alcohol consumption, and educational level were evaluated, they did not show statistically significant effect on MN frequency, Comet tail length and telomere length ($P > 0.05$). The ethnicity effect was evaluated among three races of Malay, Chinese and Indian. Ethnicity showed a statistically significant effect on MN frequency ($P = 0.004$). Working time was evaluated based on more or less than 5 years. Working time demonstrated statistically significant effect on MN frequency ($P = 0.001$), Comet tail length ($P = 0.001$) and relative telomere length ($P = 0.001$) (Tables 6, 7 and 8).

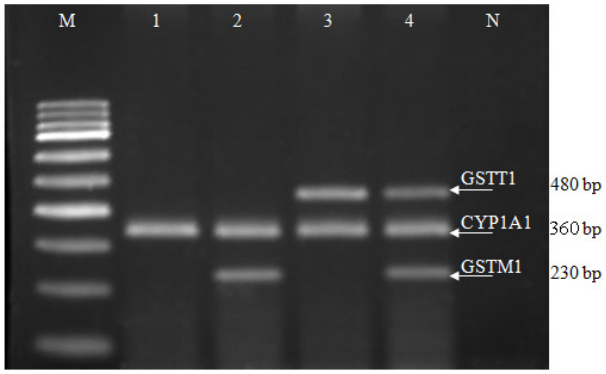

Figure 1. Multiplex PCR amplification of GSTM1 and GSTT1 was resolved on 2% agarose gel electrophoresis in samples 1-4. *Lane M* = a 100-bp DNA ladder. *Lane N* = a negative control. CYP1A1 was used as internal control.

Table 1. Presence or absence of GSTT1 and GSTM1 in the individuals studied.

Genes		Non-null (Presence)	Null (Absence)
GSTM1	Workers	67 (55.83%)	53 (44.17%)
	Controls	109 (90.83%)	11 (9.17%)
GSTT1	Workers	46 (38.33%)	74 (61.67%)
	Controls	45 (37.5%)	75 (62.5%)

Table 2. Influences of GSTM1 and GSTT1 on cell damage biomarkers between the workers and controls.

			N	Micronucleus	Tail length	Telomere length
				P value	P value	P value
GSTM1	Non-null	Workers	67	0.001	0.001	0.002
		Controls	109			
	Null	Workers	53	0.001	0.117	0.379
		Controls	11			
GSTT1	Non-null	Workers	46	0.001	0.001	0.015
		Controls	45			
	Null	Workers	74	0.001	0.001	0.012
		Controls	75			

Significant at the 0.05 level using the independent *t*-test.

Table 3. Influences of GSTM1 and GSTT1 on cell damage biomarkers in the workers and controls.

	N	MN		Tail length		Telomere length	
		Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value
Workers							
GSTM1 null	53	11.83 ± 3.90	0.289	24.34 ± 8.30	0.390	0.23 ± 0.57	0.389
GSTM1 non-null	67	12.66 ± 4.66		25.75 ± 9.33		0.32 ± 0.58	
GSTT1 null	74	11.96 ± 4.26	0.279	24.40 ± 8.96	0.261	0.26 ± 0.51	0.644
GSTT1 non-null	46	12.84 ± 4.46		26.29 ± 8.73		0.31 ± 0.67	
Controls							
GSTM1 null	11	3.82 ± 2.23	0.008	20.16 ± 7.41	0.246	1.71 ± 5.36	0.747
GSTM1 non-null	109	2.30 ± 1.72		17.21 ± 8.04		2.39 ± 6.74	
GSTT1 null	75	2.52 ± 2.06	0.490	18.19 ± 8.52	0.214	2.54 ± 7.67	0.610
GSTT1 non-null	45	2.31 ± 1.34		16.30 ± 6.98		1.98 ± 4.39	

Significant at the 0.05 level using the independent *t*-test.

Table 4. Results of age and cell damage biomarkers under influence of the GSTM1 gene.

Groups	Age (years)	G	N	Micronucleus		Tail length		Telomere length	
				Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value
Workers	≥30	N	22	14.74 ± 3.70	0.121	30.61 ± 6.48	0.585	0.03 ± 0.03	0.129
		NN	32	16.20 ± 3.45		31.96 ± 8.22		0.02 ± 0.02	
	<30	N	31	9.76 ± 2.45	0.368	19.89 ± 6.39	0.554	0.36 ± 0.72	0.205
		NN	35	9.43 ± 2.96		20.07 ± 6.14		0.59 ± 0.71	
	All	N	53	11.83 ± 3.89	0.426	24.34 ± 8.30	0.514	0.23 ± 0.57	0.387
		NN	67	12.66 ± 4.66		25.75 ± 9.33		0.32 ± 0.58	
Controls									
	≥30	N	3	6.33 ± 2.18	0.921	28.12 ± 2.93	0.921	0.13 ± 0.12	0.274
		NN	8	6.50 ± 2.12		27.83 ± 6.13		1.83 ± 2.44	
	<30	N	8	2.88 ± 1.41	0.044	17.17 ± 6.21	0.667	2.31 ± 6.29	0.960
		NN	101	1.97 ± 1.17		16.37 ± 7.58		2.44 ± 6.98	
	All	N	11	3.82 ± 2.23	0.008	20.16 ± 7.41	0.208	1.71 ± 5.36	0.747
		NN	109	2.30 ± 1.72		17.21 ± 8.04		2.40 ± 6.74	

Significant at the 0.05 level using the Mann-Whitney U-test and the independent *t*-test. G = genotypes (N = null; NN = non-null).

Table 5. Results of age and cell damage biomarkers under influence of the GSTT1 gene.

Groups	Age (years)	G	N	Micronucleus		Tail length		Telomere length	
				Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value
Workers	≥30	N	29	15.63 ± 3.67	0.903	31.27 ± 8.38	0.788	0.03 ± 0.02	0.225
		NN	25	15.57 ± 3.58		31.57 ± 6.56		0.02 ± 0.03	
	<30	N	45	9.59 ± 2.62	0.973	19.98 ± 6.11	0.923	0.40 ± 0.62	0.199
		NN	21	9.59 ± 2.99		19.99 ± 6.57		0.65 ± 0.89	
	All	N	74	11.96 ± 4.26	0.259	24.40 ± 8.96	0.144	0.26 ± 0.51	0.642
		NN	46	12.84 ± 4.46		26.29 ± 8.73		0.31 ± 0.67	
Controls	≥30	N	9	6.74 ± 2.02	0.327	27.40 ± 5.76	0.327	1.36 ± 2.36	0.979
		NN	2	5.17 ± 2.12		30.18 ± 1.67		1.41 ± 1.87	
	<30	N	66	1.95 ± 1.23	0.329	16.93 ± 8.08	0.662	2.70 ± 8.12	0.608
		NN	43	2.17 ± 1.17		15.66 ± 6.43		2.00 ± 4.48	
	All	N	75	2.52 ± 2.06	0.879	18.19 ± 8.52	0.348	2.54 ± 7.67	0.653
		NN	45	2.31 ± 1.34		16.30 ± 6.98		1.98 ± 4.39	

Significant at the 0.05 level using the Mann-Whitney U-test and the independent *t*-test. G = genotypes (N= null; NN = non-null).

Table 6. Result of micronucleus biomarker changes with the socio-demographic factors.

Group	Workers		Controls		All subjects		P value
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	
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	
Non-smokers	61	12.70 ± 0.89	90	3.01 ± 1.02	151	7.85 ± 0.72	0.494
Educated	24	13.59 ± 1.31	105	3.41 ± 0.75	129	8.50 ± 0.86	
Non-educated	96	12.80 ± 0.55	15	2.81 ± 1.59	111	7.80 ± 0.91	0.590
Drinkers	12	13.51 ± 1.13	3	3.42 ± 1.25	3	8.47 ± 1.00	
Non-drinkers	108	12.88 ± 0.65	117	2.79 ± 0.90	117	7.83 ± 0.46	0.520
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 >5 years	37	14.38 ± 0.93[a]		-			
Working time <5 years	83	11.18 ± 0.79[b]		-			0.001

Means with different superscript letters are significant at $P < 0.05$.

Table 7. Result of Comet tail length biomarker changes with the socio-demographic factors.

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

Means with different superscript letters are significant at $P < 0.05$.

Table 8. Result of telomere length shortening biomarker with socio-demographic factors.

Group	Workers (N)	Controls (N)	All subjects (N)	P
All subjects	120[a]	120[b]	240	0.046
Smokers	59	30	89	0.377
Non-smokers	61	90	151	
Educated	24	105	129	0.437
Non-educated	96	15	111	
Drinkers	12	3	3	0.683
Non-drinkers	108	117	117	
Malay	65	78	143	0.814
Chinese	45	33	78	
Indian	10	9	19	
Working time >5 years	37[a]			0.001
Working time <5 years	83[b]			

Subjects with different superscript letters are significant at $P < 0.05$.

DISCUSSION

Many xenobiotic substances are activated or detoxified by metabolizing enzymes. The genetic polymorphisms in GST enzymes could cause interindividual variability in the genotoxic damages induced by xenobiotics. Therefore, in the assessment of these enzymes it is particularly important to notice the individual genetics that are involved (Laffon et al., 2003). GSTs are located on chromosomes 1p13.3 and 22q11.2 (Movafagh et al., 2005). GSTs are polymorphic enzymes involved in the conjugation of reactive chemical intermediates with important roles in detoxification of endogenous and exogenous compounds. GSTM1 and GSTT1 genes belong to this family. GSTM1 and GSTT1 deletion polymorphisms represent null alleles. Individuals with null alleles do not metabolize the activated mutagenic compounds due to a lack of enzyme activity, which consequently increase the risk of DNA damage (Movafagh et al., 2005; Laffon et al., 2006; Heuser et al., 2007; da Silva et al., 2008). Many studies have explored the influence of lack of GSTM1 (Pavanello and Clonfero, 2000) and GSTT1 (Li et al., 2000, 2001; de Waart et al., 2001; Buzio et al., 2003; Masetti et al., 2003; Olshan et al., 2003) genes in the genotoxic damage and the biomarkers of MN (Heuser et al., 2007) and telomere length (Christiansen et al., 2006; Sainger et al., 2009). Since, genotoxic evalua-

tion is one of the necessary tests to assess the environmental quality and occupational health (Martino-Roth et al., 2002), null or non-null GSTM1 and GSTT1 genotypes were investigated to show their possible influences on the genetic material damage in occupational exposure. In these series of investigations, the increased knowledge of the workers is aimed to decrease the genetic damage and risk of major diseases (Martino-Roth et al., 2002).

In this study, the subjects were randomly selected, and data were interpreted, while taking into consideration certain factors. It was targeted to assess the possible influence of GSTM1 and GSTT1 on the biomarkers of MN, Comet tail length and telomere length in buccal cells due to occupational exposure. There are a range of compounds with potential cytotoxic effects in workplaces. Synergistic effect of compounds has been expected to cause genotoxic damage in cells in mechanical workshop workers (Heuser et al., 2007).

As we know, ageing is related to the loss of efficacy in DNA repair mechanisms, which results in accumulation of genetic lesions (Laffon et al., 2002). The increase of Comet tail length, MN frequency (Heuser et al., 2007) and shortening telomere length (Nordfjall et al., 2005; Njajou et al., 2007; Hewakapuge et al., 2008) resulting from genetic damages and DNA lesions could be considered as ageing biomarkers. Taking together, the effects of polymorphisms and ageing on the background level of the biomarkers showed the relative effect of lack of GSTT1 (Ada et al., 2007) and GSTM1 on early ageing. This could be attributed to the workers' ancestries and inheritance of unfavorable versions of genes, which increase the effects of reactive metabolites to the environmental mutagens (Laffon et al., 2002).

Difference in genetic inheritance affects the background level of biomarkers as regards to environmental and lifestyle factors. GSTM1 enzyme protects cells against the endogenous compounds of oxidative stress, including epoxides, redox-cycling products, and carbonyls (Habdous et al., 2004). GSTT1 enzyme shows both detoxifying and activating properties in environmental pollutant exposure (Pavanello and Clonfero, 2000). Lack of GSTM1 enzyme increases oxidative DNA damage on hazard exposure (Habdous et al., 2004). The detoxifying role of the GSTT1 enzyme prevents the induced genotoxic attacks on DNA (Pavanello and Clonfero, 2000). Nevertheless, the interpretation of results in this study should be treated with caution due to the insufficient sample size in each group of genotypes and ages at the younger or older groups. Regarding the possible influence of the genotypes and their interactions on the level of biomarkers, detection of polymorphisms will be of particular importance, which renders the assays more sensitive and specific to identify the effects of exposure and sensitivity of subgroups (Heuser et al., 2007). Smoking habits, alcohol consumption, working time, and ethnic factors interfere with the genotype influences, which induce increased or decreased biomarkers. The statistically nonsignificant effect of educational level suggested the protective effects of knowledge on genomic damage and ageing changes by improvement of lifestyle (Pavanello et al., 2005) (Tables 6, 7, 8). The important role of time of exposure was shown by the statistically significant effect of working time on the damaged biomarkers. However, effects of these genotypes on early ageing have to be confirmed with a suitable sample size in each group of genotypes and ages.

CONCLUSION

This study showed the possible influence of GSTM1 null genotype as a risk factor to induce genotoxic damages, irrespective of interfering factors. The results represented the

protective effects of these enzymes in respect to ageing acceleration. However, these findings should be considered as preliminary results and improved through further investigations with a larger sample size in each group of genotypes and ages. The study represented interplay of xenobiotic exposures and genetic variability in the genomic damage biomarkers. It helps to identify individuals susceptible to early ageing. Further studies in this area are needed to improve the knowledge of ageing about the causes of early ageing. This would lead to prevent the consequences of ageing.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the cooperation of all volunteers who participated in this study.

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