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Does *CYP1A1* gene polymorphism affect cell damage biomarkers and ageing?

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Abstract: This study aimed to investigate the effects of cytochrome P450 1A1 (*CYP1A1*) gene polymorphism on early ageing due to occupational exposure to mutagens. The project was conducted on buccal epithelial cells collected from mechanical-workshop workers and controls. Epithelial cells were obtained from the inner part of the cheeks. Techniques including comet assay, micronuclei test, real-time polymerase chain reaction, and restriction fragment length polymorphism-polymerase chain reaction were used to assess DNA damage biomarkers. The results showed that there was a significant difference in relative telomere length ($P = 0.039$) between wild and mutated genotypes among all individuals. Furthermore, occupational exposure showed no statistically significant effects on biomarkers of the possible influences of gene polymorphism and different rates of ageing among workers ($P > 0.05$), except for relative telomere length ($P = 0.041$) among workers as a whole. It was concluded that the effects of occupational exposure to mutagens on genetic material in buccal cells mediated by *CYP1A1* gene polymorphism were not significant. In addition, *CYP1A1* gene polymorphism might have a protective effect against premature ageing due to occupational exposure to mutagenic chemicals.

Key words: Ageing, buccal cells, *CYP1A1*, occupational exposure, restriction fragment length polymorphism

1. Introduction

Ageing occurs via the coordinated action of products produced by multiple genes (Vijg, 2000) and the combination effects of both exogenous and endogenous factors, although some elements have more importance than others (Semsei, 2000). The lifelong effects of endogenous, environmental, and occupational factors may induce DNA damage and subsequent ageing, resulting in tissue atrophy, neoplasms, loss of body functions (Vijg, 2000), and death (Smith et al., 2001).

Cytochrome P450 (CYP)-dependent reactions convert exogenous compounds to both active and inactive states (Waki et al., 2010). CYP enzymes can contribute to oxidation of xenobiotics and phase I metabolism reactions, which may increase the generation of highly reactive metabolites with possible mutagenic properties (Pande et al., 2008). In addition, genetic polymorphisms of enzymes can increase the susceptibility of genomic damage in cells by regulating the metabolism of mutagens and mediating their conversion to active forms in carrier populations (Paz-y-Mino et al., 2004).

CYP1A1 is an enzyme of the CYP group and a key enzyme in catalyzing oxidative reactions and activating xenobiotics to mutagenic reactive metabolites (Sam et al., 2008). The common polymorphism type of the *CYP1A1* gene is CYP1A1m1, which is formed by T/C substitution located 264 bp downstream from the 3'-flanking region (Li et al., 2008). Induction of the *CYP1A1* gene can lead to oxidative stress via the generation of reactive oxygen species (Stohs, 1990; Fadhel et al., 2002; Ramadass et al., 2003) and the suppression of telomerase activity as a consequence (Santos et al., 2006; Indran et al., 2010). Lack of telomerase can induce a progressive shortening in telomeric ends (Artandi and DePinho, 2010).

Telomere shortening (Shay et al., 2001) and reduced telomerase activity (Artandi and DePinho, 2010) can promote premature ageing and degenerative disorders. It is known that telomerase activity, telomere length, and cell growth can be initially decreased followed by an increase upon occupational exposure to mutagens, which happens through increased levels of both *CYP1A1* gene expression and oxidative stress (Perumal Kuppusamy, 2012). DNA biomarkers can play roles in identifying genetic polymorphisms (Bardakcı, 2001), which indicate different sequences of nucleic acid at a particular location in the genome (Surgun et al., 2012). Furthermore, using biological parameters can increase the ability to detect DNA damage and can highlight the potential health risks of some chemicals to which there is long-term exposure (Martino-Roth et al., 2002). Thus, this study

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aimed to evaluate the effects of occupational exposure to mutagens on early ageing under the influence of *CYP1A1* polymorphism using DNA damage biomarkers.

2. Materials and methods

Approval and permission for the study were obtained from the ethics committee of the Medical and Health Sciences Faculty [Reference Number: UPM/FPSK/PADS/T7-MJKEtikaPer/F01 (JSB-Aug (08)05)]. Samples were buccal mucosa epithelial cells that were collected from 120 workers who were exposed to mutagenic chemicals in mechanical workshops and from 120 controls who had not been exposed. Subjects were interviewed about their health status, educational level, smoking habits, alcohol consumption, work history, duration of employment, and other aspects relevant to the study. In addition, duration of employment was assessed, and subjects were divided into 2 groups of more or less than 5 years of employment.

Respondents were asked to rinse their mouth with water before collection of samples. After that, cells were collected by scraping the inner part of both cheeks with a cytology brush. Cells were gently mixed with 0.9% sodium chloride and phosphate-buffered saline in separate microcentrifuge tubes and brought to the laboratory. Micronuclei test (MNT), comet assay, restriction fragment length polymorphism (RFLP), and real-time polymerase chain reaction (PCR) were used to assess the effects of occupational exposure to mutagens on buccal cells under the influence of *CYP1A1* gene polymorphism. Methods used to evaluate the biologic parameters included micronucleus (MN) formation, comet tail length, and relative telomere length. The MNT and comet assay followed similar protocols described in a previous paper (Eshkoor et al., 2011). Genomic DNA was extracted from the cells using a QIAamp DNA Blood Mini Kit (QIAGEN, France). The extracted DNA was run on a 0.7%–1% agarose gel and quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA). This extracted DNA was used for real-time PCR and RFLP.

2.1. Real-time PCR

Real-time PCR was used to measure telomere length. Primers used in the study were telomere and 36B4, as described by Cawthon (2002). Primer sequences were: tel1, GGTTTTTGAGGGTGAG GGTGAG GGTGAGGGTGAGGGT; tel2,TCCCGACTATCCCTA TCCCTATCCCTATCCCTATCCCTA; 36B4u, CAGCAA GTGGGAAGGTGTAATCC; and 36B4d, CCCATTCTA TCATCAACGGGTACAA.

The ratio of telomere repeat copy number to single gene copy number (T/S) was determined using the Corbett Rotor-Gene 6000 (Corbett Life Science, Australia) with 36 wells. For PCR reaction, a 25μL volume of solution was prepared in PCR tubes. In each run, both gene telomere and 36B4 were placed for each sample in separate tubes. Primers were purchased from Bioline Inc. (UK). The solution for PCR reaction included 0.6 μL of each primer, 1 μL of EvaGreen, 1 μL of DNA, 5 μL of ImmoMix master mix (Bioline), and 16.8 μL of pure water. The PCR reaction proceeded as 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 °C to 95 °C. The data obtained from samples were interpreted through a dataset to assess threshold cycle values. The T/S ratio was calculated based on the fractional number between the average 36B4 Ct value and the average telomere Ct value for each sample. One sample was used as a reference in each run in triplicate for comparing results. The formula of $2^{-\Delta\Delta}$ was used to calculate the T/S ratio. For this calculation, the formula $\Delta C_T = C_t$ (telomere) – C_t (36B4) was applied. In addition, the final calculation was based on the results of the ΔC_t (target) – ΔC_t (reference) formula to compare obtained measurements and determine the proper T/S ratio. A serial dilution of genomic DNA derived from one sample was run in order to have a good view of the efficiency of the PCR reaction standard curve. The PCR product sizes for telomere and 36B4 were 76 and 74 bp, respectively.

2.2. PCR reaction and RFLP

The RFLP method was applied to identify *CYP1A1* gene polymorphism. First, the PCR reaction was performed to optimize the primers used. The forward and reverse primers were described in a previously published article (Cha et al., 2007). Primer sequences for *CYP1A1* were: sense, 5'-CAGTGAAGAGGTGTAGCCGCT-3', and antisense, 5'-TAGGAGTCTTGTCTGATGCCT-3'. The solution volume in the PCR tube was 25 μL, which included 5 μL of ImmoMix master mix, 0.6 μL of primer, 2 to 6 μL of genomic DNA, and 12.8 to 16.8 μL of pure water. The ImmoMix master mix (Bioline) for PCR reactions contained dNTPs, Taq polymerase, $MgCl_2$, and a buffer. Tubes were placed in a G-Storm Thermal Cycler (Gene Technology Ltd., UK) for PCR reaction. The PCR reaction was performed for 35 cycles.

The first cycle was initiated with incubation at 94 °C for 5 min. After that, the PCR reaction followed with a denaturation step at 94 °C for 30 s, annealing at 62 °C for 30 s, extension at 72 °C for 45 s, and final extension at 72 °C for 10 min after the last cycle. The proper annealing temperature was obtained by gradient PCR analysis. After the completion of the reaction, samples were stored at 4 °C until use. A negative control without DNA template was carried out in each run. The specific PCR product was identified by running 1.8%–2% agarose gel electrophoresis and was viewed with an AlphaImager analysis system (Alpha Innotech, USA). The PCR product size was 360 bp. After the reaction was finished, 15 mL of product was put

aside for RFLP. First, 7.2 µL of ddH2O was added to 0.2 mL of volume in the PCR tube, and then mixed with 1.5 µL of restriction enzyme buffer and 6.0 µL of the amplified PCR product. At the final step, 0.3 µL of the Msp1 restriction enzyme at 10 units/µL (Fermentas, USA) was added to the tube.

The mixture was gently pipetted up and down to mix the solution well. The RFLP reaction mixture was incubated at 37 °C for 16 h. Next, the enzyme was inactivated by incubation in a heating block at 80 °C for 20 min. Finally, the sizes of the products were identified using agarose gel electrophoresis. DNA ladders of 50 and 100 bp (Bioline) were used to identify the sizes of the products in RFLP. Gels used for PCR and RFLP products were 2% and 4%, respectively. Products were viewed under UV light using the AlphaImager TM 2200 system.

2.3. Statistical analysis

The data were analyzed using SPSS 16.0 (SPSS Inc., USA). The statistical tests used were the independent t-test and the Mann–Whitney U test. MN frequency and DNA damage tail length were examined using the nonparametric Mann–Whitney U test. The analysis method for relative telomere length was the independent t-test. Hardy–Weinberg equilibrium was evaluated using the chi-square test, which is a goodness-of-fit test. The critical level for rejection of the null hypothesis (2-tailed test) was a P-value of 5% (P = 0.05).

3. Results

The *CYP1A1* gene was amplified by PCR followed by RFLP using the Msp1 restriction enzyme. Figures 1 and 2 are images of PCR and RFLP products of the *CYP1A1* gene. After amplification, the product was 360 bp in size (Figure 1). The RFLP products of the *CYP1A1* gene using the Msp1 restriction enzyme were of the A1A1 (360 bp), A1A2 (360, 220, and 140 bp), and A2A2 (220 and 140 bp) genotypes (Figure 2). These products were identified as wild and mutated. The A1A1 genotype was placed in

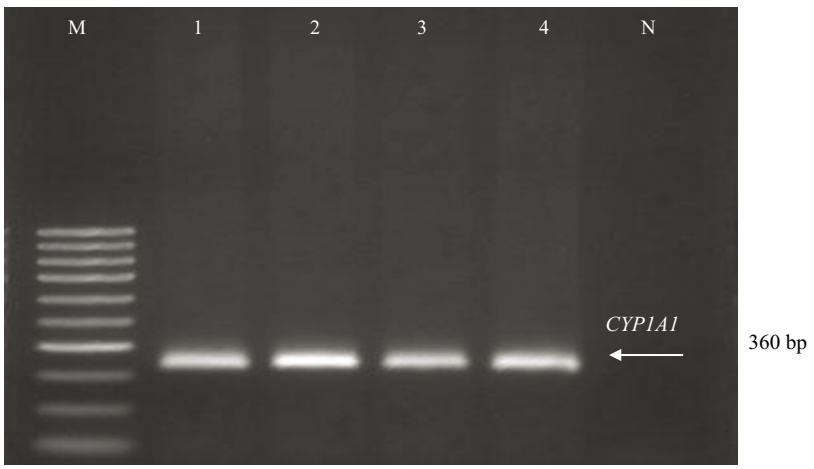

Figure 1. The PCR product of the *CYP1A1* gene was resolved in 2% agarose gel electrophoresis in samples 1–4. M represents 100 bp DNA ladder. N is negative control during PCR reaction.

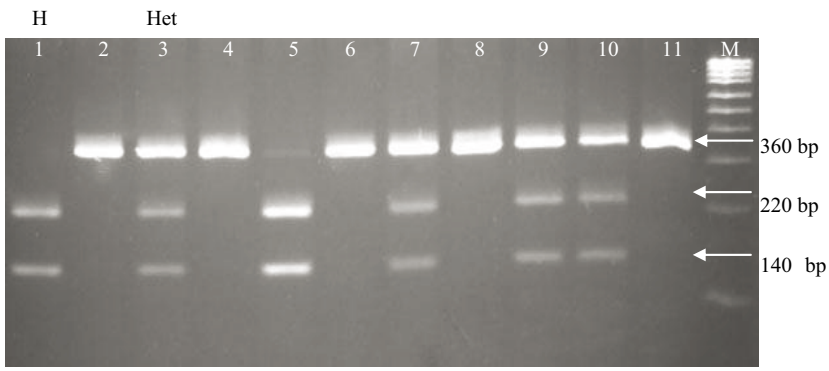

Figure 2. The restriction fragments for *CYP1A1* gene resolved in 4% agarose gel electrophoresis in samples 1–11. M represents 100 bp DNA Ladder. H and Het are homozygote and heterozygote genotypes, respectively.

the wild group, and the A1A2 and A2A2 genotypes were in the mutated group. Frequencies of A1A1, A1A2, and A2A2 genotypes in the entire group were 45.0%, 46.7%, and 8.3%, respectively. Differences in MN and comet tail length between wild and mutated genotypes were not statistically significant in individuals (P > 0.05), except for relative telomere length (P = 0.039) (Table 1).

The workers carrying wild or mutated genotypes showed a significantly higher MN frequency and shorter telomere length compared to controls (P < 0.05). In addition, neither genotype showed any statistically significant effects on comet tail length (P > 0.05) (Table 2). Workers and controls were divided by age into 2 groups, below 30 years and over 30 years. None of them showed statistically significant biomarker changes under the effects of gene polymorphism and age (P > 0.05), except for relative telomere length (P = 0.041) in workers (Table 3). The findings of sociodemographic factors indicated that ethnicity had a significant effect on MN frequency (P = 0.004). Furthermore, employment duration of greater than 5 years significantly affected MN frequency (P = 0.001), comet tail length (P = 0.001), and relative telomere length (P = 0.001). The findings indicated that smoking, alcohol consumption, and educational level had no statistically significant effects on any of the biomarkers (P > 0.05).

4. Discussion

Several chemicals used or generated in mechanical workshops are classified as mutagens. These substances include gasoline, diesel, butane gas, styrene, benzene, and chloroform (Paz-y-Mino et al., 2008). The statistical association between different genotypes and both higher MN frequency and shorter telomere length among workers was probably due to the interplay between genotypes and chemicals, which can cause genotoxic effects and DNA damage (Quiñones et al., 2006). As previous investigations have reported (Lautraite et al., 2002; Paz-y-Mino et al., 2008), the *CYP1A1* gene was not able to cause DNA damage and therefore failed to form a comet tail in damaged cells. Such findings can be explained by the insufficient effect of this gene on comet tail length (Lautraite et al., 2002; Moretti et al., 2007; da Silva et al., 2008; Paz-y-Mino et al., 2008; Villarini et al., 2008), MNs (Norppa, 1997; Ishikawa et al., 2004), or both (Pavanello and Clonfero, 2000; Paz-y-Mino et al., 2004).

Our study showed that the *CYP1A1* gene did not contribute to the ageing process. It was found that *CYP1A1* gene polymorphism did not affect MN frequency, comet tail length, or telomere length in different age groups, except for telomere length in workers as a whole. It indicated a possible protective effect of the gene against ageing, which was probably due to age-related transcriptional activity changes (Sy et al., 2001). These changes can increase resistance to genotoxic damage, ageing, and mediation of age-related patterns (Keshava et al., 2005). In addition, such protection could be a result of a combination with phase II enzymes, which may contribute to the detoxification of potential internal and external mutagens. Apparently,

Table 1. The effects of *CYP1A1* gene polymorphism on cell damage biomarkers in all individuals studied.

CYP1A1 genotypes	n	MN	Comet tail length	Relative telomere length
		P-value	P-value	P-value
A1A1	108	0.320	0.919	0.039
(A1A2, A2A2)	132			

The Mann–Whitney U test for DNA damage and MN and the independent t-test for telomere length were used (P = 0.05).

Table 2. The effects of different genotypes of *CYP1A1* on biomarkers between workers and controls.

CYP1A1 genotypes	Groups	N	MN		Comet tail length		Relative telomere length	
			Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
A1A1	Workers	56	12.71 ± 4.68	0.001	24.99 ± 9.14	0.057	0.17 ± 0.22	0.028
	Controls	52	2.49 ± 1.82		17.47 ± 8.40		1.17 ± 3.24	
(A1A2, A2A2)	Workers	64	11.93 ± 4.03	0.001	25.24 ± 8.72	0.122	0.37 ± 0.75	0.006
	Controls	68	2.40 ± 1.83		17.49 ± 7.74		3.22 ± 8.24	

The Mann–Whitney U test for DNA damage and MN and the independent t-test for telomere length were used (P = 0.05).

Table 3. The *CYP1A1* gene polymorphism and age effects on the biomarkers between mutated and wild genotypes in workers and controls.

Groups	Age	G*	n	MN		Comet tail length		Relative telomere length	
				Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
Workers	≥30	W	28	15.72 ± 4.25	0.401	31.21 ± 7.83	0.822	0.02 ± 0.02	0.311
		M	26	15.49 ± 2.80		31.63 ± 7.34		0.03 ± 0.03	
	<30	W	28	9.70 ± 2.78	0.800	18.78 ± 5.38	0.410	0.31 ± 0.23	0.065
		M	38	9.50 ± 2.71		20.88 ± 6.69		0.60 ± 0.91	
	All	W	56	12.71 ± 4.68	0.414	24.99 ± 9.14	0.782	0.17 ± 0.22	0.041
		M	64	11.93 ± 4.03		25.24 ± 8.72		0.37 ± 0.75	
Controls	≥30	W	7	5.71 ± 1.67	0.058	26.02 ± 5.14	0.131	1.59 ± 2.63	0.672
		M	4	7.75 ± 2.15		31.21 ± 4.23		0.97 ± 1.37	
	<30	W	45	1.99 ± 1.25	0.759	16.14 ± 8.05	0.584	1.10 ± 3.35	0.056
		M	64	2.07 ± 1.19		16.63 ± 7.09		3.36 ± 8.47	
	All	W	52	2.49 ± 1.82	0.755	17.47 ± 8.40	0.970	1.17 ± 3.24	0.061
		M	68	2.40 ± 1.83		17.49 ± 7.74		3.22 ± 8.24	

The Mann–Whitney U test for DNA damage and MN and the independent t-test for telomere length were used (P = 0.05).
*G = Genotypes (W = wild genotype, M = mutated genotype).

the impact of the *CYP1A1* gene on genomic damage is associated with the level of corresponding enzyme activity (Georgiadis et al., 2005), the combination of genes, and their interactions (Xue et al., 2001; Lee et al., 2008). In contrast to our results, several studies have shown effects of the *CYP1A1* gene on ageing (Smith et al., 2001) and DNA damage due to environmental and occupational hazards exposure (Sy et al., 2001; Taioli et al., 2003; Pande et al., 2008). The influence of gene polymorphism on telomere length in the workers studied suggests that a partial effect of the gene on ageing is likely because of the possible effects of enzyme activity and genotoxicity (Leng et al., 2004). *CYP1A1* gene polymorphism induction due to occupational exposure to mutagens can possibly affect the variations of telomere length in genomic DNA (Bin et al., 2011), which is possibly due to the contribution of the gene to cell cycle progression (Kalmes et al., 2011).

However, the results should be interpreted carefully because of the possible effects of confounding factors, including gene and polymorphism interactions as well as sample sizes for each genotype and age group (Georgiadis et al., 2005). For example, sociodemographic factors can alter the influence of gene polymorphism on the genome (Garte et al., 2006; Kirsch-Volders et al., 2006). In the current study, significant difference in MN frequency among ethnic groups could be related to a higher prevalence of specific gene polymorphism in an ethnic group, which increases susceptibility to genomic damage (Zhan et al., 2010).

It was concluded that MN and telomere length shortening were sensitive biomarkers to express induced genotoxicity resulting from the possible influence of the *CYP1A1* gene due to occupational exposure to mutagenic chemicals. Our results suggest that *CYP1A1* gene polymorphism possibly has protective effects against ageing. Thus, analysis of genotypes and related enzymatic activities can be useful in identifying the risk of genomic damage in workers who are occupationally exposed to mutagens. However, further research is required to determine the exact role of the *CYP1A1* gene and its related genotypes in DNA damage and acceleration or retardation of the ageing process.

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