RESEARCH ARTICLE

CYP1A1 Gene Polymorphisms: Modulator of Genetic Damage in Coal-Tar Workers

Shiv Kumar Giri[1], AnitaYadav[1]*, Anil Kumar[1], Kapil Dev[1], Sachin Gulati[1], Ranjan Gupta[2], Neeraj Aggarwal[3], Sanjeev Kumar Gautam[1]

Abstract

Aim: It is well known that polycyclic aromatic hydrocarbons (PAHs) such as benzo (a) pyrene have carcinogenic properties and may cause many types of cancers in human populations. Genetic susceptibility might be due to variation in genes encoding for carcinogen metabolizing enzymes, such as cytochrome P-450 (*CYP450*). Our study aimed to investigate the effect of genetic polymorphisms of *CYP1A1* (*m1* and *m2*) on genetic damage in 115 coal-tar workers exposed to PAHs at their work place. Methods: Genetic polymorphisms of *CYP1A1* were determined by the PCR-RFLP method. Comet and buccal micronucleus assays were used to evaluate genetic damage among 115 coal tar workers and 105 control subjects. Results: Both *CYP1A1 m1* and *CYP1A1 m2* heterozygous and homozygous (*wt*/*mt*+*mt*/*mt*) variants individually as well as synergistically showed significant association (P<0.05) with genetic damage as measured by tail moment (TM) and buccal micronuclei (BMN) frequencies in control and exposed subjects. Conclusion: In our study we found significant association of *CYP1A1 m1* and *m2* heterozygous (*wt*/*mt*)+homozygous (*mt*/*mt*) variants with genetic damage suggesting that these polymorphisms may modulate the effects of PAH exposure in occupational settings.

Keywords: PAHs - genetic damage - tail moment - coal-tar workers - *CYP1A1* - buccal micronuclei

Asian Pacific J Cancer Prev, **13**, 3409-3416

Introduction

Coal-tar workers, workings at coal-tar melting stations and road construction sites routinely expose themselves to a large number of physical or chemical genotoxic agents such as polycyclic aromatic hydrocarbons (PAHs) and heterocyclic compounds by inhalation and dermal absorption at their work place (King et al., 1984). PAHs are human mutagens and carcinogens, commonly found adhering to airborne particulate matter and a complex mixture of more than 100 compounds (Boström et al., 2002). Benzo (a) pyrene [B (a) P] is perhaps the most well studied PAHs and was recently classified as a human carcinogen (IARC, 2009). Many epidemiological studies have shown an increased risk of lung, stomach, non-melanoma skin cancers, and leukemia in occupational workers with high exposure of PAHs from coal-tar products (Hansen, 1992; Wong et al., 1992; Partanen and Boffetta, 1994).

Monitoring of biological effects as a measure of the internally effective dose is more relevant for assessment of the ultimate health risks such as cancer. Many biomarkers such as chromosomal aberrations, micronuclei, sister chromatid exchanges and comet assay have been developed to estimate exposure and to assess in an early phase the risk of adverse health effects (Wogan, 1992; Van Delft et al., 1998). Micronuclei (MN) index in human cells has become one of the standard cytogenetic endpoints and biomarkers used in genetic toxicology in vivo or ex vivo (Holland et al., 1999). It originates from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division (Fenech et al., 2002). In humans, MN can be easily assessed in erythrocytes, lymphocytes, and exfoliated epithelial cells (e.g. oral, urothelial, nasal) to obtain a measure of genome damage induced in vivo. Primary DNA damage is considered to be an important initial event in carcinogenesis. The comet assay (single-cell gel electrophoresis) has become the preferred test for the qualitative and quantitative assessment of DNA damage in single cells and capable of detecting DNA single- and double-strand breaks, alkali-labile sites and incomplete excision repair sites, and genomic structural discontinuities (Singh et al., 1988; Collins, 2004).

Attention has been recently focused on genetic polymorphisms that seem appear to modulate human exposure to genotoxic insult (Norppa, 2004). Cytochrome P-450 (*CYP450*) and glutathione *S*-transferases (*GST*) genes are two important classes, encoding carcinogen metabolizing enzymes, involved in the metabolism of

*[1]Department of Biotechnology, [2]Department of Biochemistry, [3]Department of Microbiology Kurukshetra University, Kurukshetra, Haryana, India *For correspondence: ayadav@kuk.ac.in*

carcinogens like PAHs (Wormhoudt et al., 1999). The members of subfamily 1 of the *CYP* gene super family play a major role in the catalysis of such metabolic activation (Nebert, 1991). Among these, *CYP1A1* is a key enzyme which catalyzes oxidative reactions and activates xenobiotics like B (a) P to carcinogenic reactive metabolites, thereby involving in the pathogenesis of various malignancies (Gajecka et al., 2005). It encodes an aromatic hydrocarbon hydroxylase enzyme that catalyzes the oxidation of PAHs to their phenolic metabolite or diol epoxides (Bartsch et al., 2000). *CYP1A1* contains four functional polymorphisms. *CYP1A1 m1* and *m2* are the most studied polymorphism as they either effect the activation or induction of enzyme. *CYP1A1* m1 (MspI) is T→C transition located downstream of exon 7 (Cascorbi et al., 1996). It does not exert any effect on *CYP1A1* induction but increases the microsomal enzyme activity. *CYP1A1 m2* (Ile-Val), A→G transition leads to an amino-acid substitution of Val for Ile in exon 7 and is significantly associated with *CYP1A1* inducibility (Crofts et al., 1994). *CYP1A1* polymorphisms have been shown to be associated with moderate to high risk of lung cancer in Asians (Hayashi et al., 1991) and in Caucasian and Hawaiian population (Kawajiri et al., 1990; Marchand et al., 1998) making it a strong candidate gene in susceptibility to smoking-related cancers of larynx, mouth, esophagus, urinary bladder and kidney (Doll, 1998). A strong positive association of *CYP1A1 m1* and *CYP1A1 m2* polymorphisms with lung cancer risk has been found in human population (Houlston, 2000; Song et al., 2001; Sobti et al., 2004). Therefore, it is reasonable to hypothesize that genetic polymorphisms of *CYP1A1* may play a role in individual susceptibility to genetic damage and developing various types of cancers. The *CYP1A1* gene polymorphisms were examined extensively to evaluate the possible role they play in DNA damage and cancer promotion in PAHs exposed populations (Chen et al., 2006; Moretti et al., 2007). Coal-tar workers, working at coal-tar melting and road construction sites, are poorly characterized regarding the *CYP1A1* gene polymorphisms as a modulator of genetic damage. Therefore, the present study was planned. Moreover, there are both positive and negative findings regarding the effect of genetic polymorphism of *CYP1A1* on genetic damage in PAHs exposed population, which further warranted this study.

Materials and Methods

Subject and sampling

The studied population included 115 coal-tar workers who worked at the coal- tar melting stations and at road constructions sites and spend daily >8 h in working environment. Control subjects (105) were healthy volunteers who were not engaged in coal-tar melting and road construction works and have socio-economic status matched to exposed workers. All the subjects enrolled in our study were of Asian origin, living in rural and sub urban area of Haryana state in India. They were informed about the study before obtaining consent. An approval for the study was taken from the institutional human ethics committee of Kurukshetra University, Kurukshetra (Haryana). A standard questionnaire was used to assess standard demographic characteristics such as age, sex, exposure duration and consumption habits (smoking, alcohol use and tobacco chewing) and medical history. The person who smokes tobacco through cigarette or bedi in routine life was considered as smoker while the people who never smoke tobacco through cigarette or bedi in routine life is considered as non smoker. The person who takes alcohol in routine life was considered as alcohol user while the person who never takes alcohol in routine life was considered as non alcohol user. Subjects undergoing medical treatment, X-ray exposure, radiography or vaccination up to 3 months before sampling have not been included. As far as possible, control subjects were being matched with respect to age, sex and consumption habits. These individuals were not occupationally exposed to PAHs.

Sample collection

Blood (about 0.5 mL) samples were collected from exposed workers and control subjects in disposable pre-sterilized and K2EDTA coated vaccutainer tubes (Medikit, India) with the help of a trained technician. Buccal samples were collected on pre-cleaned slides with wooden spatula. Urine samples were collected in 50 mL autoclaved centrifuge tubes. All samples were brought to laboratory in a well insulated ice box. Urine samples were stored at -20°C until analysis of 1-hydroxy pyrene (1-OHP).

Urinary 1-hydroxy pyrene analysis for internal PAH exposure

To assess the PAHs exposure in exposed population, the level of 1-OHP in random urine samples of exposed workers and control subjects was analyzed by standard method (Jongeneelen et al., 1987) using gas chromatography (Perkin Elmer Auto system XL) with flame ionizing detector from Indian Institute of Toxicology Research (IITR), Lucknow (U.P), India.

DNA damage analysis by single cell gel electrophoresis (comet assay)

Alkaline comet assay was performed according to the method of Singh et al. (1988) and Tice et al. (2000) with minor modifications. Slides were prepared in duplicate per sample. Dust free, plain slides was covered with a layer of 150 μL of 1% normal melting agarose (NMA) and allowed to dry for 10 min in hot air oven. This layer served as an anchor for additional layers to prevent slippage. The blood sample (5-10 μL) was mixed with 90 μL of warm 0.5% low melting agarose (LMA) and this mixture was layered as second additional layer and gelled at 4°C for 15 min. A third additional layer of 150 μL of 0.5% LMA was added on top and gelled again at 4 °C for 15 min. The slides were treated for 2 hours at 4 °C in freshly prepared, chilled lysis buffer solution (25 mM NaCl, 100 mM sodium EDTA, 10 mM tris. 1% triton X -100, 10 % DMSO added before use and pH adjusted to 10). They were removed from the lysis solution, incubated in alkaline electrophoresis buffer (10N NaCl, 200 mM EDTA, pH adjusted to 13) for 20 min followed by electrophoresis (25 V and 300 mA) for 30

min, in the same buffer. The slides were then neutralized with tris buffer (0.4 M tris, pH adjusted to 7.5), rinsed with distilled water and stained with 100 μL ethidium bromide (20 μg mL-1) for 5 min. under dim or dark condition.

Comet Scoring

A total of 50 cells from each of the duplicate slides were examined randomly under fluorescence microscope. The extent of DNA damage was measured quantitatively as tail moment (TM) using comet assay IV software. The TM is defined by the percentage of DNA in the tail multiplied by the length between the center of the head and tail (Olive et al., 1990).

Buccal micronuclei (BMN) in exfoliated epithelial cells

Buccal cells originate from a multilayered epithelium that lines the oral cavity. Prior to buccal cell collection, the coal-tar workers and control subjects 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 smears on glass slides were transported to laboratory in insulated ice box and processed within 3-4 h of sample collection. The samples were air dried and hydrolyzed for 8 min. in 1N HCl at 60ºC. After a rinse in tap water, slides were stained in Aceto-orecine (Hi-Media, India) for 10 minutes at room temperature. Then samples were given a brief washing in ethanol and distilled water. Counter staining was done with fast green solution (Hi-Media, India) for 12 minutes followed by final rinse in ethanol and distilled water. Slides were air dried and screened in a double blind manner to analyze the presence of micronuclei. At least 1000 cells were scored for presence of micronuclei in exfoliated buccal cells at 1000 X magnification under bright field microscope. The scoring of micronuclei was done according to criterion of Tolbert et al. 1991.

CYP1A1 m1 and CYP1A1 m2 genotyping

Genomic DNA was isolated from 200 μL of whole blood by Spin column kit (Bangalore genei, India). *CYP1A1* genotypes at the *m1* and *m2* sites were analyzed by PCR-based restriction fragment length polymorphism (RFLP) method (Song et al., 2001) with minor modifications. The primers for the *m1* and *m2* site (M1F 5'-AAGAGGTG TAGC GCTGCACT-3', M1R 5'-TAGGAGTC TCTCATGCCT-3' and M2F 5'-TTC CAC CCG TTG CAG CAG GAT AGC C-3', M2R 5'-CTG TCT CCC TCT GGT TAC AGG AAG-3') generate 340 and 204 bp fragments respectively. These fragments were amplified separately but under the same conditions as follows: a 25 μl reaction mixture consisted of ~100 ng template DNA, 10 μM each primer, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 1.0 U Taq DNA polymerase with 1X reaction buffer (Bangalore Genei, Bangalore, India) and 5% dimethyl sulfoxide. Amplification was performed in a eppendorf gradient thermocycler .To amplify the fragment containing the m1site, the PCR profile consisted of an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 61°C for 1 min, elongation at 72°C for 1 min and a final elongation step at 72°C for 10 min. The fragment containing the *m2* sites was amplified using the PCR conditions which included initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 63°C for 30s, extension at 72°C for 30s and final elongation step at 72°C for 10 min. The restriction enzyme MspI (Fermentas) was used to distinguish the m1polymorphism; gain of MspI restriction site occurs in the polymorphic allele. The wild-type allele has a single band representing the entire 340 bp fragment and the variant allele results in two fragments of 200 and 140 bp. The restriction enzyme *BsrDI* (Fermentas) was used to detect *m2* polymorphism. Loss of *BsrDI* site occurs in polymorphic allele which gives a single band of 204 bp whereas the wild-type alleles generate 149 and 55bp bands (for the *m2* site). The restricted product was analyzed by electrophoresis in 3% agarose gel containing ethidium bromide (Figure 1A-1B).

Statistical analysis

The student t test was used for comparison of age between studied groups. χ^2 test was applied for difference in sex, consumption habits and exposure history among studied population. The influence of *CYP1A1* polymorphism on studied biomarkers among multiple sub-groups was done by post hoc analysis using ANOVA and Mann Whitney-U test. The interaction of different confounding factors such as age, gender, consumption habits, exposure and *CYP1A1 m1* and *m2* genotypes with TM value and BMN frequency was studied using linear regression model. All of the statistical analysis was performed with SPSS.16. The level of significance was set at 0.05.

Results

General demographic characteristics of population

As shown in Table. 1, no significant (P > 0.05)

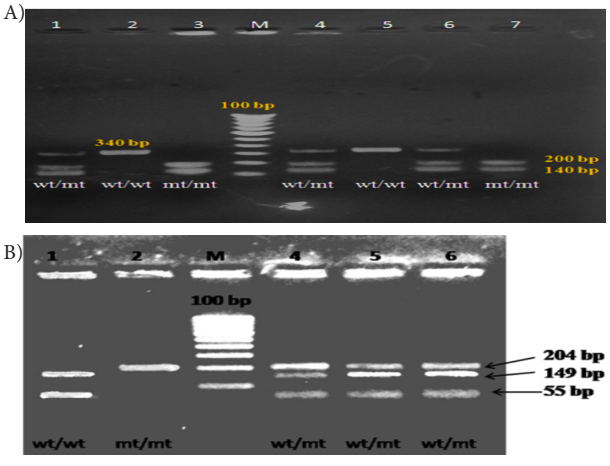

Figure 1. Ethidium Bromide Stained 3% Agarose Gel . A) *CYP1A1 m1* Polymorphism by PCR-RFLP. Lane M shows 100bp molecular weight (MW) marker, lane 2 and 5 represent homozygous wild-type alleles, lane 1, 4 and 6 represents heterogous allele, lane 3 and 7 represent homozygous mutant-type allele. B) *CYP1A1 m2* Polymorphism by PCR-RFLP. Lane M shows 100bp molecular weight (MW) marker, lane 4, 5 and 6 represent heterozygous mutant type allele, lane 1 represents wild type allele and lane 2 represents homozygous mutant type allele.

Table 1. Demographic Characterization of Control and Exposed Population

Variable		Control	Exposed	OR	95%CI	P value
Total (N)		105	115			
Age** (Year)		37.33±10.00	35.72±9.94			>0.05
Age groups, N (%):	≤ 45	81 (71.10)	98 (85.20)	0.585	0.29-1.16	0.12
	≥ 45	24 (22.90)	17 (14.80)			
Sex, N (%):	Male	67 (63.80)	80 (69.60)	0.771	0.44-1.45	0.36
	Female	38 (36.20)	35 (30.40)			
Smoking status, N (%):	Smoker	39 (37.10)	52 (45.20)	0.716	0.41-1.22	0.22
	Never smoker	66 (62.90)	63 (54.80)			
	Cigarette or bidi pack/week**	4.03±1.22	4.13±0.99			
Alcoholic status, N (%)	Alcohol user	41 (39.00)	47 (40.90)	0.844	0.49-1.43	0.53
	Non alcohol user	64 (61.00)	68 (59.10)			
	Alcohol quantity** (ml/week)	386.21±83.21	395.12±72.2			
Tobacco chewing status, N (%):	Tobacco chewer	41 (39.00)	47 (40.90)	0.927	0.54-1.59	0.78
	Never Tobacco chewer	64 (61.00)	68 (59.10)			
	Tobacco pouch/week**	3.98±1.06	4.50±1.28			
PAH exposure (%):	≤ 25 Year	84 (73.00)				
	≥ 25 Year	31 (27.00)				<0.05

*Student t-test was applied for comparing mean value of age among control and exposed group. SD- Standard deviation, χ^2 test was applied for difference in sex and consumption habits exposure history among studied population. OR- Odd ratio, CI- Confidence interval. ** Mean±SD

Table 2. DNA Damage and BMN Frequency

CYP1A1 genotypes	Control-105			Exposed-115		
	N (%)	Tail Moment (μM) (Mean ± SD)	BMN/1000 BE Cells (Mean ± SD)	N (%)	Tail Moment (μM) (Mean ± SD)	BMN/1000 BE Cells (Mean ± SD)
Influence of *CYP1A1* polymorphisms						
CYP1A1 m1 *wt/wt*	57 (54.30)	0.32 ± 0.22	4.59 ±1.93	50 (43.50)	11.96 ± 0.53	6.62±1.33
wt/mt+mt/mt	48 (45.70)	0.60 ± 0.34*¥	5.66 ±1.81*¥	65 (56.50)	12.14 ± 0.56*¥	7.47±1.64*¥
CYP1A1 m2 wt/wt	66 (62.90)	0.40 ± 0.28	4.86 ±1.93	59 (51.30)	11.95 ± 0.50	6.71±1.47
wt/mt+mt/mt	39 (37.10)	0.53 ± 0.35*¥	5.46 ±1.74*¥	56 (48.70)	12.18 ±0.59*¥	7.51±1.56*¥
Synergistic effect of *CYP1A1 m1* and *CYP1A1 m2 CYP1A1 m1+m2*						
CYP1A1m1+m2 wt/wt	42	0.31 ± 0.20	4.52 ± 1.80	47	11.86 ± 0.48	6.59±1.34
CYP1A1 m1 wt/wt with *CYP1A1 m2 wt/mt+mt/mt*	15	0.36 ± 0.28	4.80 ± 1.89	3	11.92 ± 1.17	7.00±1.73
CYP1A1 m1 wt/mt+mt/mt with *CYP1A1 m2 wt/wt*	24	0.55 ± 0.33	5.45 ± 2.06	11	12.13 ± 0.37	7.27±1.95
CYP1A1 m1 and *m2 wt/mt+mt/mt*	24	0.64 ± 0.36*	5.87 ± 1.57*	54	12.23 ± 0.56*	7.51±1.56*

*Significant at P<0.05, Multivariate ANOVA test was used for the comparison of Tail moment and BMN frequency in multiple subgroups among studied population, ¥Significant at P<0.05+high mean rank (Mann-Whitney U test), SD- Standard deviation.

difference was found in the distributions of age, sex and consumption habits between the 115 coal- tar workers and the 105 control subjects. Genetic damage was assessed by alkaline comet assay in term of TM value in peripheral blood lymphocytes and MN frequency in exfoliated buccal epithelial cells.

Urinary 1 - Hydroxy pyrene assessment

The mean concentration of 1-OHP in random urine samples of control and exposed subjects were found to be 0.029±0.010 μg mL^{-1} and 0.304±0.23 μg mL^{-1} respectively . The level of 1-OHP in urine of exposed subjects was found significantly higher than that of control subjects (P<0.05).

Influence of CYP1A1 polymorphism on genetic damage

In the present study, the effect of *CYP1A1* polymorphisms on genetic damage was studied in control and exposed subjects. Significant association (P<0.05) of the *CYP1A1 m1* and *CYP1A1 m2* heterozygous and homozygous (*wt/mt+mt/mt*) variants with TM value and BMN frequency as compare to wild type (wt/wt) genotype was found in both control and exposed subjects (Table 2).

Synergistic effect of CYP1A1 m1 and m2 polymorphism

As shown in Table 2, both *CYP1A1 m1* and *m2* heterozygous and homozygous (*wt/mt+mt/mt*) variants synergistically showed significant association (P<0.05) with genetic damage (TM value and BMN frequency) among the studied population.

Interaction of confounding factors, exposure and CYP1A1 genotypes with genetic damage

By linear regression analysis, we studied the interaction

Table 3. Linear Regression Model for Interaction of Sex, Age, Consumption Habits, Exposure and *CYP1A1* Polymorphisms

	Exposed					
Model	Un-standardized Coefficient (B)	Standardized Coefficient (β)	R^2	t	95%CI	P Value
DNA damage: TM(Constant)						
Age	0.96	0.12	0.04	1.28	-0.05-0.24	0.2
Sex	0.2	-0.171	0.02	-1.84	0.42-0.01	0.06
Smoking	-0.16	-0.296	0.08	-3.29	-0.26-(-0.06)	0.07
Alcoholic	-0.26	-0.466	0.21	-5.59	-0.35-(-0.16)	0.06
Tobacco chewing	0.08	0.124	1.32	-2.21	-0.04 to 0.21	0.19
Exposure	-0.25	-0.453	0.2	-5.4	-0.35-(-0.16)	0.03
CYP1A1 m1	0.18	0.199	0.03	2.15	0.01-0.34	0.03
CYP1A1 m2	0.24	0.079	0.07	3.05	0.08-0.39	0
BMN frequency: BMN(Constant)						
Age	-0.324	-0.136	0.322	-1.15	-0.87-0.23	0.251
Sex	0.33	0.091	0.357	1.07	-0.28-0.94	0.286
Smoking	0.082	0.049	0.256	0.57	-0.19-0.36	0.565
Alcoholic	-0.286	-0.172	0.345	-1.78	-0.60-031	0.077
Tobacco chewing	-0.229	-0.136	0.377	-1.3	-0.57-0.11	0.195
Exposure	1.444	0.701	0.484	5.88	0.95-1.93	0
CYP1A1 m1	0.412	0.183	0.055	2.25	0.05-0.77	0.026
CYP1A1 m2	0.405	0.155	0.035	2.31	0.05-0.75	0.022

* 'Models using stepwise method and adjusting by tail moment. CI: confidence interval, R^2: correlation coefficient, Models excluding control individuals. Significant at P<0.05.

of *CYP1A1* genotypes, confounding factors and exposure with TM value and BMN frequency. We observed that *CYP1A1 m1* and *CYP1A1 m2* genotypes and exposure showed significant effect (P<0.05) on TM value and BMN frequency while other factors such as age, sex and consumption habits did not show any significant (P>0.05) association with BMN frequency and TM value among studied population (Table 3, data of control population are not shown).

Discussion

Many biomarkers have been developed to estimate exposure and to assess in an early phase the risk of adverse health effects (Wogan, 1992; Van Delft et al., 1998). These can be measured in exhaled air, blood, urine and in tissue samples. The actual target organ or cell is usually not available for measurements and biomarkers of exposure are thus often surrogate measures of doses or effects at the target. The ideal biomarker has been described as chemical-specific, detectable at low (trace) levels, available using non-invasive techniques, inexpensive to analyse and quantitatively related to prior exposures (Henderson et al., 1989). Thus, for biomonitoring purposes, biological materials should be easily accessible in sufficient amounts under routine conditions and without unacceptable discomfort and health risk for the individual. For these reasons blood and urine are most commonly used and cells in blood may provide surrogate endpoints for effects in internal organs (Angerer et al., 2007). Buccal cells are the first barrier of the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products (Auturp et al., 1985). Approximately 90% human cancers originated from epithelial cells (Rosin et al., 1992).Therefore, it could be argued that oral epithelial cells represent a preferred target site for early genotoxic event induced by carcinogenic agents entering the body via inhalation and ingestion.

To predict PAHs exposure in exposed subjects, we analyzed 1-OHP level in random urine samples of exposed and control subjects. The level of urinary 1-OHP was found significantly higher among PAHs exposed coal-tar workers than in matched controls. This finding is consistent with the results of other studies aimed to determine the effects of occupational exposure to PAHs on urinary 1-OHP concentrations (Ovrebo et al., 1994; Dell'Omo et al, 1998; Nan et al., 2001; Siwinska et al., 2004). In our previous study, we assessed the mean concentration of benzo (a) pyrene in ambient air (biomarker of external exposure) at working site of coal-tar/road construction workers. It was found to be 10.71±3.45 ng m^{-3} which is quite high when compared to the concentration of B (a) P in ambient air (Kumar et al., 2011).

The contradictory reports are available regarding the association of confounding factors such as age, sex and consumption habits (smoking, alcohol intake and tobacco chewing) with genetic damage as primary DNA damage and BMN frequency. Some biomonitoring studies found positive association of age (Ozkul et al.,1997; Moretti et al., 2000), sex (Betti et al., 1994; Bajpayee et al., 2002; Fenech et al., 2003), smoking (Burgaz et al., 1999; Hoffmann et al., 2005), alcohol intake (Ramirez et al., 2002) and tobacco chewing (Nair et al., 1991; Trivedi et al., 1993) while some studies observed no association of age (Zhu et al., 1999; Konapaka et al., 2003), sex (Anderson et al., 1993; Zhu et al, 1999; Konapaka et al., 2003), smoking (Moller et al., 2006), alcohol intake (Celik et al., 2007; Martinez-Valenzuela et al., 2009) with genetic damage. In our study, we did not observe any significant association of age, sex and consumption habits (smoking and alcohol use) with genetic damage.

CYP1A1 polymorphism is often considered to be associated with enhanced inducibility, leading to higher enzymatic activity that activates pre carcinogens like PAHs which cause DNA damage. *CYP1A1* alleles (*m1* and *m2*) are known as "higher risk alleles" and frequencies of these alleles are reported to be eight to eighteen times higher in Asians than in Caucasian population (Bartsch et al., 2000). A number of studies reported increased risk of lung cancer in individuals with smoking habit and at least a single mutant allele of *CYP1A1* in Asian population (Song et al., 2001; Sobti et al., 2004). In our findings *CYP1A1 m1* and *CYP1A1 m2* heterozygous and homozygous (*wt/mt+mt/mt*) variants individually as well as synergistically were significantly associated with genetic damage as compared to *CYP1A1 m1* (*wt/wt*) and *CYP1A1 m2* (*wt/wt*) wild type alleles in PAHs exposed coal-tar workers and control subjects. To the best of our knowledge we did not find any study showing association of *CYP1A1* gene polymorphism with MN in exfoliated buccal epithelial cells in PAHs exposed population. As in our results *CYP1A1* variant genotype has been found to be associated with higher levels of DNA damage in

different occupational workers such as coke-oven workers (Brescia et al., 1999; Rojas et al., 2000), pot-room workers (Carstensen et al., 1999), traffic policemen (Carere et al., 2002). In our previous study we observed significant association between GSTM1 and GSTT1 null genotypes and BMN frequencies among coal-tar population (Kumar et al., 2011). However, contrary to our results, some studies reported that *CYP1A1 m1* and *m2* variants did not show any effect on DNA damage among coke-oven and graphide electrode workers (Marczynski et al., 2002; Chen et al., 2006; Moretti et al, 2007). Mahimkar et al. (2010) found no association of *CYP1A1* gene polymorphism with MN in exfoliated buccal cells in leukoplakia patients.

In conclusion, in our study we found significant association of *CYP1A1 m1* and *m2* heterozygous (*wt/mt*)+homozygous (*mt/mt*) variants with genetic damage as assessed by TM value and BMN frequency in studied population suggesting that this polymorphism may modulate the effects of PAHs exposure in occupational settings. However, the underlying mechanisms of this observed effect modification, and their related consequences, remain to be further investigated before this finding can be applied to monitoring individuals susceptible to the PAHs-induced carcinogenesis. More studies are needed for confirming the role of *CYP1A1 m1* and *CYP1A1 m2* as biomarker of susceptibility and for the better understanding of gene-environmental exposure interactions

Acknowledgements

This study was proposed and guided by Dr. Anita Yadav. The work was carried out in Animal Biotechnology Laboratory, Department of Biotechnology, Kurukshetra University, Kurukshetra (Haryana), India and funds for research were provided by Department of Science and Technology (DST), New Delhi, India. We are thankful to all the blood donors for this study. The authors declare no conflict of interest.

References

Anderson D, Francis AJ, Godbert P, Jenkinson PC, Butterworth KR (1993). Variability in chromosome aberrations, sister chromatid exchanges, and mitogen-induced blastogenesis in peripheral lymphocytes from control individuals. *Environ Health Perspect*, **104**, 83-8.

Angerer J, Ewers U, Wilhelm M (2007). Human biomonitoring: state of the art. *Int J Hyg Environ Health*, **210**, 201-28.

Autrup H, Seremet T, Arenholt D, Dragsted L, Jepsen A (1985). Metabolism of benzo[a]pyrene by cultured rat and human buccal mucosa cells. *Carcinogenesis*, **6**, 1761-5.

Bartsch H, Nair U, Risch A, et al (2000). Genetic polymorphism of *CYP* genes, alone or in combination, as a risk modiWer of tobacco-related cancers. *Cancer Epidemiol Biomarkers. Prev*, **9**, 3-28.

Bajpayee M, Dhawan A, Parmar D, et al (2002). Gender-related differences in basal DNA damage in lymphocytes of a healthy Indian population using the alkaline comet assay. *Mutat Res*, **520**, 83-91.

Betti C, Davini T, Giannessi L, Loprieno N, Barale R (1994). Microgel electrophoresis assay (Comet test) and SCE analysis in human lymphocytes from 100 normal subjects. *Mutat Res*, **307**, 323-33.

Boström CE, Gerde P, Hanberg A, et al (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect*, **110**, 451-88.

Brescia G, Celotti L, Clonfero E, et al (1999). The influence of Cytochrome P450 1A1 and glutathione S-transferase M1genotypes on biomarker levels in coke-oven workers. *Arch Toxicol*, **73**, 431-9.

Burgaz S, Karahalil B, Bayrak P, Taskin L, Yavuzaslan F, Bokesoy I, Anzion RB, Bos RP, Platin N (1999). Urinary cyclophosphamide excretion and micronuclei frequencies in peripheral lymphocytes and in exfoliated buccal epithelial cells of nurses handling antineoplastics. *Mutat Res*, **439**, 97-104.

Carstensen U, Hou SM, Alexandrie AK, et al (1999). Influence of genetic polymorphisms of biotransformation enzymes on gene mutations, strand breaks of deoxyribonucleic acid, and micronuclei in mononuclear blood cells and urinary 8-hydroxydeoxyguanosine in potroom workers exposed to polyaromatic hydrocarbons. *Scand J Work Environ Health*, **25**, 351-60.

Carere A, Andreoli C, Galati R, et al (2002). Biomonitoring of exposure to urban air pollutants: analysis of sister chromatid exchanges and DNA lesions in peripheral lymphocytes of traffic policemen. *Mutat Res*, **518**, 215-24.

Cascorbi I, Brockmoller J, Roots I (1996). A C4887A polymorphism in exon 7 of human *CYP1A1*: population frequency, mutation linkages and impact on lung cancer susceptibility. *Cancer Res*, **56**, 4965-9.

Celik M, Donbak L, Unal F, et al (2007). Cytogenetic damage in workers from a coal-fired power plant. *Mutat Res*, **627**, 158-63.

Chen YY, Bye J, Yuang W, et al (2006). Association of polymorphisms in AhR, *CYP1A1*, GSTM1 and GSTT1 genes with levels of DNA damage in peripheral blood lymphocytes among coke-oven workers. *Cancer Epidemiol. Biomarkers Prev*, **15**, 1703-7.

Collins AR (2004). The comet assay for DNA damage and repair: Principles, applications, and limitations. *Mol Biotechnol*, **26**, 249-61.

Crofts F, Taioli E, Trachman J, et al (1994). Functional significance of different human *CYP1A1* genotypes. *Carcinogenesis*, **15**, 2961-3.

Dell'Omo M, Muzi G, Marchionna G, et al (1998). Preventive measures reduce exposure to polycyclic aromatic hydrocarbons at a graphite electrode plant. *Occup Environ Med*, **55**, 401-6.

Doll R (1998). Uncovering the risk of smoking: historical perspective. *Stat Meth Med Res*, **7**, 87-117.

Fenech M, Crott JW (2002). Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes-evidence for breakagefusion- bridge cycles in the cytokinesis-block micronucleus assay. *Mutat Res*, **504**, 131-6.

Fenech M, Bonassi S, Turner J, et al (2003). Intra- and interlaboratory variation in the scoring of micronuclei and nucleoplasmic bridges in binucleated human lymphocytes: results of an international slide-scoring exercise by the HUMN project. *Mutat Res Genet Toxicol Environ Mutagen*, **534**, 45-64.

Houlston RS (2000). *CYP1A1* polymorphisms and lung cancer risk: a meta-analysis. *Pharmacogenetics*, **10**, 105-14.

Jongeneelen FJ, Anzion RBM, Henderson PTh (1987). Determination of hydroxylated polycyclic aromatic hydrocarbons in urine. *J Chrom*, **413**, 227-32.

Gajecka M, Rydzanicz M, Jaskula-Sztul R, et al (2005). *CYP1A1*, CYP2D6, CYP2E1, NAT2, GSTM1 and GSTT1 polymorphisms or their combinations are associated with the increased risk of the laryngeal squamous cell carcinoma. *Mutat Res*, **574**, 112-23.

Hansen E (1992). Cancer mortality and incidence in mastic asphalt workers. *Scand J Environ Health*, **18**, 133-41.

Hayashi SI, Watanabe J, Nakachi K, Kawajiri K (1991). PCR detection of an A/G polymorphism within exon 7 of the *CYP1A1* gene. *Nucleic Acids Res*, **19**, 47-97.

Henderson R, Bechtold WE, Bond JA, Sun JD, (1989). The use of biological markers in toxicology. *Crit Rev Toxicol*, **20**, 65-82.

Hoffman H, Speit G (2005). Assessment of DNA damage in peripheral blood of heavy smokers with the comet assay and the micronucleus test. *Mutat Res*, **581**, 105-14.

Holland MN, Chang WP, Zeiger E, Bonassi S (1999). The Human Micro- Nucleus Project—an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutat Res*, 428, 271-283.

Jongeneelen FJ, Anzion RBM, Henderson PTh (1987). Determination of hydroxylated polycyclic aromatic hydrocarbons in urine. J. Chrom, 413, 227-32.

IARC (2009). Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum 92 (in press).

Kawajiri K, Nakachi K, Imai K, Yoshii A, Shinoda N, Watanabe J, (1990). Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of cytochrome P4501A1 gene. FEBS Lett, 263, 131-133.

King RW, Puzinauskas VP, Holsworth CE. (1984). Asphalt composition and health effects: a critical Review. American Petroleum Institute Technical Publication,Washington DC, 1-11.

Konopacka M (2003). Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells. Neoplasma, 50, 380-382.

Mahimkar MB, Tanuja A, Kannan SS, Patil T (2010). Influence of genetic polymorphisms on frequency of micronucleated buccal epithelial cells in leukoplakia patients. Oraloncology, 46 (10), 761-766

Marczynski B, Rihs HP, Rossbach BJ, Lzer HO, Angerer J, Scherenberg M, Bruning G, Wilhelm M (2002). Analysis of 8-oxo-7,8-dihydro-20-deoxyguanosine and DNA strand breaks in white blood cells of occupationally exposed workers: comparison with ambient monitoring, urinary metabolites and enzyme polymorphisms. Carcinogenesis, 23, 273-281.

Marchand Le, Sivaraman L, Pierce L, Seifried A, Lum A, Wilkens LR, Lau AF (1998). Association of *CYP1A1*, GSTM1 and CYP2E1 polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. Cancer Res, 58, 4858-4863.

Martinez-Valenzuela C, Gomez-Arroyo S, Villalobos-Pietrini R, Waliszewski S, Calderon-Segura ME, Felix-Gastelum R et al (2009). Genotoxic biomonitoring of agricultural workers exposed to pesticides in the north of Sinaloa State, Mexico. Environ Int, 35, 1155-9.

Moretti M, dell'Omo M, Villarini M et al (2007). Primary DNA damage and genetic polymorphisms for *CYP1A1*, EPHX and GSTM1in workers at a graphite electrode manufacturing plant. BMC Public Health, 7, 270-279.

Moretti M, Villarini M, Scassellati Sforzilini G, Pasquini R (2000). Pesticide-induced primary DNA damage in peripheral blood leukocytes of farm workers evaluated by the computerized "comet" assay. Biomarkers, 5, 192-204.

Nan HM, Kim H, Lim HS, et al (2001). Effects of occupation, lifestyle and genetic polymorphisms of *CYP1A1*, CYP2E1, GSTM1 and GSTT1 on urinary 1 - hydroxypyrene and 2 naphthol concentrations. *Carcinogenesis*, **22**, 787-93.

Nair U, Obe G, Nair J, et al (1991). Evaluation of frequency of micronucleated oral mucosa cells as a marker for genotoxic damage in chewers of betel quid with or without tobacco, *Mutat Res*, **261**, 163-8.

Nebert DW (1991). Role of genetics and drug metabolism in human cancer risk. *Mutat Res*, **247**, 267-81.

Norppa H (2004). Cytogenetic biomarkers and genetic polymorphisms. *Toxicol Lett*, **149**, 309-34.

Olive PL, Banath JP, Durand RE (1990). Heterogeneity in radiation induced DNA damage and repair in tumor and normal cells using the comet assay. *Radiat Res*, **122**, 86-94.

Ovrebo S, Haugen A, Fjeldstad PE, Hemminki K, Szyfter K (1994). Biological monitoring of exposure to polycyclic aromatic hydrocarbon in an electrode paste plant. *J Occup Med*, **36**, 303-10.

Ozkul Y, Donmez H, Erenmemisoglu A, Demirtas H, Imamoglu N (1997). Induction of micronuclei by smokeless tobacco on buccal mucosa cells of habitual users, *Mutagenesis*, **12**, 285-7.

Partanen TJ, Boffetta P (1994). Cancer risk in asphalt workers and roofers: review and meta-analysis of epidemiologic studies. *Am J Ind Med*, **26**, 721-40.

Ramirez A, Saldanha PH, (2002). Micronucleus investigation of alcoholic patients with oral carcinomas. *Genet Mol Res*, **1**, 246-60.

Rojas E, Valverde M, Lopez MC, et al (2000). Evaluation of DNA damage in exfoliated tear Duct epithelial cells from individuals exposed to air pollution assessed by single cell gel electrophoresis assay, *Mutat Res*, **468**, 11-7.

Rosin M P (1992). The use of the micronucleus test on exfoliated cells to identify anticlastogenic action in humans: a biological marker for the efficacy of chemopreventive agents. *Mutat Res*, **267**, 265-76.

Singh NP, McCoy MT, Tice RR, Schneider EL (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res*, **175**, 184-91.

Siwinska ED, Mielzynska L, Kapka (2002). Association between urinary 1-hydroxypyrene and genotoxic effects in coke oven workers. *Occup Environ Med*, **61**, 10.

Sobti RC, Sharma S, Joshi A, Jindal SK, Janmeja A (2004). Genetic polymorphism of the *CYP1A1*, CYP2E1, GSTM1 and GSTT1 genes and lung cancer susceptibility in a North Indian population. *Mol Cell Biochem*, **266**, 1-9.

Song N, Tan W, Xing D, Lin D (2001). *CYP1A1* polymorphism and risk to lung cancer in relation to tobacco smoking: a case-control study in China. *Carcinogenesis*, **22**, 11-6.

Tice RR, Agurell E, Anderson A, et al (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. *Environ Mol Mutagen*, **35**, 206-21.

Tolbert PE, Shy CM, Allen JW (1991). Micronucleus and other nuclear anomalies in buccal smears: a field test in snuff users. *Am J Epidemiol*, **134**, 840-50.

Trivedi AH, Dave BJ, Adhvaryu SG (1993). Monitoring of smokeless tobacco consumers using cytogenetic endpoints. *Anticancer Res*, **13**, 2245-9.

Van Delft, JHM, Roza L, Baan RA (1998). Biological effect markers for exposure to carcinogenic compound and their relevance for risk assessment. *Crit Rev Toxicol*, **28**, 477-510.

Wogan GN (1992). Molecular epidemiology in cancer risk assessment and prevention: recent progress and avenues for future research. *Env Hlth Perspect*, **98**, 167-78.

Wong O, Bailey WJ, Amsel J (1992). Cancer mortality and incidence in mastic asphalt workers. *Scand J Environ Health*, **18**, 133-41.

Wormhoudt LW, Commandeur JN, Vermeulen NP (1999).

Genetic polymorphisms of human N-acetyltransferase, cytochrome P450, glutathione-S-transferase, and epoxide hydrolase enzymes: Relevance to xenobiotic metabolism and toxicity. *Crit Rev Toxicol*, **29**, 59-124.

Zhu Q, Lam TH, Jiang CQ, et al (1999). Lymphocyte DNA damage in cigarette factory workers measured by the Comet assay. *Mutat Res*, **444**, 1-6.