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Research Article

Cytochrome P450 (CYP) and glutathione S-transferases (GST) polymorphisms (CYP1A1, CYP1B1, GSTM1, GSTP1 and GSTT1) and urinary levels of 1-hydroxypyrene in Turkish coke oven workers

Ahmet Oguz Ada[1], Meltem Yilmazer[2], Sinan Suzen[1], Canan Demiroglu[2], Ali Eba Demirbag[3], Sibel Efe[4], Yavuz Alemdar[4], Sema Burgaz[2] and Mumtaz Iscan[1]

[1]*Department of Toxicology, Faculty of Pharmacy, Ankara University, Tandogan, Ankara, Turkey.*
[2]*Department of Toxicology, Faculty of Pharmacy, Gazi University, Etiler, Ankara, Turkey.*
[3]*Gastrointestinal Surgery Department, Yuksek Ihtisas Hospital, Sihhiye, Ankara, Turkey.*
[4]*Eregli Iron and Steel Works Co., Karadeniz Eregli, Turkey.*

Abstract

Genetic polymorphisms of xenobiotic metabolizing enzymes have been associated with cancer risk. We evaluated the influences of genetic polymorphisms of polycyclic aromatic hydrocarbon (PAH) metabolizing enzymes on urinary 1-hydroxypyrene (1-OHP) excretion in Turkish coke oven workers. Urinary 1-OHP was analyzed by HPLC after enzymatic hydrolysis. Lymphocyte DNA was used for PCR-based genotyping of cytochrome P450 (CYP) polymorphisms (CYP1A1 and CYP1B1) and glutathione S-transferases (GST) polymorphisms (GSTM1, GSTT1 and GSTP1). The mean urinary 1-OHP levels of coke oven workers were significantly higher than that of controls. No significant difference was detected in the mean urinary 1-OHP levels of smokers and non-smokers either for coke oven workers or controls. Genetic polymorphisms of the CYPs and GSTs studied had no significant influence on 1-OHP excretion in coke oven workers, but in the control group the urinary 1-OHP levels of individuals carrying the GSTT1- genotype were significantly higher than those of individuals carrying GSTT1+ genotype. The duration of occupational exposure and metabolic genotype for GSTT1 were the significant predictors of urinary 1-OHP levels. The control individuals carrying combined GSTM1-/GSTT1- genotypes also had significantly higher levels of urinary 1-OHP than those of individuals carrying GSTM1+/GSTTl+, GSTM1-/GSTT1+, and GSTM1+/GSTT1- genotypes. These results indicate that urinary 1-OHP is a sensitive indicator of recent human exposure to PAHs and that genetic polymorphism of GSTT1 may also to some extent reflect the interindividual variation in susceptibility to PAHs only at low PAH exposure.

Key words: polycyclic aromatic hydrocarbons, 1-hydroxypyrene, coke oven workers, cytochrome P450s, glutathione S-transferases.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are present in the environment when coal is pyrolysed into coke, because of which coke oven workers are at a high risk of exposure to PAHs (Wu *et al.*, 1998; Kuljukka-Rabb *et al.*, 2002). An epidemiological 30-year follow-up study has confirmed that occupational exposure to coke oven emissions is associated with significant excess mortality from cancer of the respiratory system and of the prostate (IARC, 1987; Binkova *et al.*, 1998).

The initial step in the metabolic activation of PAHs is mediated by cytochrome P450 (CYP) gene products, mainly isozyme CYP1A1. It has been reported that CYP1A1 polymorphism is associated with the increased formation of the ultimate DNA-binding B[*a*]P diol epoxides and an increased risk of lung cancer in smokers (Bartsch *et al.*, 1992; Bartsch *et al.*, 1995). In addition to CYP1A1 other CYP isozymes, such as CYP1B1, may also participate in the metabolic activation of PAHs, such as the metabolism of pyrene to an active epoxide (Shimada *et al.*, 1996; Kim *et al.*, 1998; Rihs *et al.*, 2005). Recent functional studies have also reported significantly higher levels of CYP1B1 in the peripheral leukocytes of lung cancer patients than controls (Wu *et al.*, 2004a). The CYP1B1 isozyme has also been shown to be polymorphic, epidemiologic studies of a possible role of this polymorphism in lung cancer having been reported by Watanabe *et al.* (2000) and Sorensen *et al.* (2005).

Send correspondence to Meltem Yilmazer. 1005 Bryant Way, Suite# N, Sunnyvale, CA 94087 USA. E-mail: meltem@lifetime.oregonstate.edu.

Many carcinogens, including PAHs, are detoxified by Phase II enzymes such as glutathione S-transferases (GSTs) (Ketterer and Mulder, 1990). The three GST classes (GSTM, GSTT and GSTP) constitute an important part of the total GST family, and well-characterized polymorphisms are known for all three classes. The GSTM1 polymorphism, which is the deletion of the GSTM1 gene leading to the absence of the enzyme, has been reported to be a moderate risk factor for the development of lung cancer (Alexandrie *et al.*, 1994). The GSTT1 polymorphism is also caused by a deletion that results in the total lack of gene product (Alexandrie *et al.*, 2000), deficiency in this enzyme having been correlated with an increased risk of lung cancer (To-Figueras *et al.*, 1997). The GSTP1 polymorphisms GSTP1 (Ile105 Val) and GSTP1 (Ala114 Val) also lead to a decrease in the activity of the corresponding enzyme (Watson *et al.*, 1998), and it has recently been shown that the GSTP1 polymorphism is associated with an increase risk of lung carcinoma (Wang *et al.*, 2003).

Pyrene is one of the most abundant PAHs in coal tar and almost exclusively metabolized to the highly fluorescent pyrene metabolite 1-hydroxypyrene (1-OHP), which accounts for about 90% of the total urinary excretion of pyrene in all species, including humans, studied thus far (Wu *et al.*, 1998). This metabolite can be quantified in urine by high pressure liquid chromatography (HPLC). In addition, urinary 1-OHP is considered to be a good biological indicator of exposure to PAHs which has the advantage of taking into account the various routes of penetration into the body (Ross *et al.*, 1997; Alexandrie *et al.*, 2000). Also, some studies show that the polymorphism of enzymes affect urinary excretion of this biological indicator (Wu *et al.*, 1998; Viezzer *et al.*, 1999; Alexandrie *et al.*, 2000).

In the study described in this paper, we evaluate the influence of polymorphisms in enzymes involved in PAH metabolism on the urinary excretion of 1-OHP of Turkish coke oven workers possibly exposed to PAH and controls who had no occupational exposure to PAH.

Materials and Methods

Subjects and specimen collection

We enrolled 100 Turkish workers in this study, the PAH exposed group consisting of 50 male workers employed in the Karadeniz Eregli iron and steel plant in the city of Eregli possibly occupationally exposed to polycyclic aromatic hydrocarbons (PAHs), and the control group consisting of 50 males from the same plant employed in packaging and other duties in which they were, presumably, not exposed to PAH. All the exposed workers used working clothes, helmets, shoes, gloves and masks during their work. All participants in the study provided their written informed consent and completed questionnaires which elicited information on demography, work history, job description, protective measures, tobacco smoking status, dietary information (alcohol consumption, fruit, grilled meat, vitamins, etc.), type of heating used at home and past and present medication. The characteristics of the population studied are summarized in Table 1. Individuals who worked for less than three months, were undergoing medical treatment, radiology or vaccination up to 3 months before sampling were not included in the study. All subjects had a mixed diet. The post-shift urine samples from the workers were collected in a PVC container without preservatives in the spring of 2001 and kept at -20 °C until analysis. Blood samples were also collected at the same time as the urine samples and were processed within 5 h of collection. The study was approved by the medical ethics board of Ankara University.

Analyses of urinary 1-OHP levels

Urinary 1-OHP was determined by an adaptation of the method of Jongeneelen *et al.* (1987). We added 20 mL of 0.1 M acetate buffer (pH 5.0) to 10 mL of urine which was then adjusted to pH 5 with 4 N hydrochloric acid and incubated for 16 h with 12.5 μL of β-glucuronidase/sulfatase at 37 °C in an electronically controlled rotary shaking bath for enzymatic hydrolysis of the conjugates. We used a C_{18} reversed-phase liquid chromatographic purification cartridge (Alltech Associates, Inc., USA) to extract PAH metabolites from urine, the cartridge being primed with 5 mL of methanol followed by 5 mL of distilled water before the hydrolyzed sample was passed through the cartridge. Subsequently, the cartridge was washed with 10 mL of distilled water and the retained solutes eluted with 9 mL of methanol. The elute was evaporated to dryness at 40 °C under nitrogen gas and the residue dissolved in methanol and subjected to HPLC with fluorescence detection at an excitation wavelength of 242 nm and an emission wavelength of 388 nm. We then injected 25 μL of the extracted sample onto a 150 x 4.6 mm inside diameter Lichrosorb RP18 (5 μm) column, the column temperature being 40 °C and the mobile phase flow rate 0.8 mL min^{-1}. The 1-OHP fraction was eluted for 5 min with water containing 57% methanol (v/v), the methanol concentration being increased

Table 1 - Descriptive data for the coke oven workers and controls.

Variables	Workers	Controls
Number of individuals (n)	50	50
Age range in years*	25 to 49	22 to 53
Mean age ± standard deviation (SD), in years*	40.42 ± 6.62	38.72 ± 9.46
Duration of exposure, range in years	1-25	
Mean duration of exposure± SD, in years	13.84 ± 6.80	
Number of non-smokers†	18 (36%)	7 (14%)
Number of non-smokers†	32 (64%)	43 (86%)

*$p > 0.05$, †$p < 0.05$.

to 69% (v/v) over the following 20 min and remaining at this level for 2 min before being returned to the initial concentration using a linear gradient function. The 1-OHP fraction peaked at 17 min. Urinary 1-OHP concentrations were corrected for creatinine concentrations and were expressed as μmol mol^{-1} creatinine.

Identification of genotypes

The DNA used for polymorphic analysis was isolated from the lymphocytes of donors by using DNA isolation kit purchased from Promega Corporation (Madison WI, USA) following the manufacturer's instructions, this company also providing the restriction enzymes used in the procedures described below.

Genetic polymorphism analysis for the CYP1A1 *m2* (CYP1A1*2B) cytochrome P4501A1 employed the PCR restriction fragment length polymorphism (PCR-RFLP) method described by Cascorbi *et al.* (1996). The CYP1A1 *Ile462Val* genetic polymorphism analysis was performed in 30 μL reaction mixture containing 25 pmol of each primer (sense 5'-CTG TCT CCC TCT GGT TAC AGG AAG C, antisense 5'-TTC CAC CCG TTG CAG CA GGA TAG CC) plus 200 μmol of each dNTPs, 3 μL of 10 X PCR buffer (100 mM Tris-HCl pH 9.0 25 °C, 500 mM KCl), 1.6 mM $MgCl_2$, 100 ng DNA and 1 unit of Taq DNA polymerase. Amplification was for 35 cycles of 0.5 min at 94 °C, 0.5 min at 63 °C and 0.5 min at 72 °C. The PCR products were digested with the *BsrD*I restriction enzyme and separated using 3% (w/v) agarose gel electrophoresis. The wild-type genotype gave bands at 55 bp and 149 bp, the heterozygous mutant genotype at 204 bp and 149 bp and the homozygous mutant genotype at 204 bp.

The polymorphism analysis for the CYP1B1 *m2* cytochrome P4501B1 genotype used the PCR-RFLP method described by Bailey *et al.* (1998). The CYP1B1 *Asn453Ser* genetic polymorphism analysis was performed in a 30 μL reaction mixture containing 25 pmol of each primer (sense 5'-GTG GTT TTT GTC AAC CAG TGG, antisense 5'-GCC CAC TGA AAA AAT CAT CAC TCT GCT GGT CAG GTG C) plus 90 μmol of each dNTP, 3 μL of 10 X PCR buffer (100 mM Tris-HCl pH 9.0 25 °C, 500 mM KCl), 1.5 mM $MgCl_2$, 500 ng DNA and 1 unit of Taq DNA polymerase. Amplification was for 35 cycles of 1 min at 95 °C, 1 min at 62 °C and 1 min at 72 °C. The PCR products were digested with the *Cac8*I restriction enzyme and digestion products separated using 3% (w/v) agarose gel electrophoresis. The wild-type genotype gave a band at 143 bp, the heterozygous mutant genotype at143 and 105 bp and the homozygous mutant genotype at105 bp.

Polymorphism analysis for the GSTM1 and the GSTT1 genes was carried out simultaneously in a single assay using a multiplex PCR approach based on the method of Abdel-Rahman *et al.* (1996). Briefly, 1 μg of isolated DNA was amplified in a 30 μL reaction mixture containing 30 pmol of each GSTM1 primer (sense 5'-GAA CTC CCT GAA AAG CTA AAG C, antisense 5'-GTT GGG CTC AAA TAT ACG GTG G) and the GSTT1 primers corresponding to the 3' coding region of human cDNA (sense 5'-TTC CTT ACT GGT CCT CAC ATC TC, antisense 5'-TCA CCG GAT CAT GGC CAG CA). As an internal control exon 7 of the CYP1A1 gene was co-amplified using appropriate primers (sense 5'-GAA CTG CCA CTT CAG C TG TCT, antisense 5'- CAG CTG CAT TTG GAA GTG CTC). The reaction mixture also contained 200 μmol of each dNTP, 3 μL of 10 X PCR buffer (100 mM Tris-HCl pH 9.0 25 °C, 500 mM KCl), 1.6 mM $MgCl_2$ and 1 unit of Taq DNA polymerase. Amplification consisted of melting at 94 °C for 5 min, followed by 35 cycles of melting at 94 °C for 2 min, annealing at 59 °C for 1 min and extension at 72 °C for 10 min. Products were separated by 3% (w/v) agarose gel electrophoresis. The presence or absence of a band at 480 bp corresponded to the presence or absence of GSTT1 and likewise for GSTM1 at 215 bp. A 312 bp band, corresponding to the CYP1A1 gene, was always present and was used as an internal control to indicate successful PCR amplification.

The polymorphism analysis for the GSTP1 (Ile105Val) genotype was determined using the PCR-RFLP method described by Park *et al.* (1999). The GSTP1 *Ile/Val* genetic polymorphism analysis was performed in a 30 μL reaction mixture containing 25 pmol of each primer (sense, 5'- AAT ACC ATC CTG CGT CAC CT, antisense 5'- TGA GGG CAC AAG AAG CCC CTT) plus 100 μmol of each dNTP, 3 μL of 10 X PCR buffer (100 mM Tris-HCl pH 9.0 25 °C, 500 mM KCl), 1.2 mM $MgCl_2$, 100 ng DNA and 1 unit of Taq DNA polymerase. Amplification was for one cycle of 2 min at 95 °C, 40 cycles of 0.5 min at 94 °C, 0.5 min at 55 °C and 0.5 min at 72 °C, followed by a final 10 min extension at 72 °C. Products were digested with the *Bsm*A1 restriction enzyme and the digestion products separated by 3% (w/v) agarose gel electrophoresis. The wild-type genotype gave bands at 305 bp and 138 bp, the heterozygous mutant genotype at 305 bp, 222 bp and 138 bp, and the homozygous mutant genotype at 222 bp and 138 bp.

Polymorphism analysis for the GSTP1 (Ala114Val) codon 114 genotype was conducted using the PCR-RFLP method described by Park *et al.* (1999). The GSTP1 *Ala/Val* genetic polymorphism analysis was performed in a 30 μL reaction mixture containing 25 pmol of each primer (sense 5'- ACA GGA TTT GGT ACT AGC CT, antisense 5'- AGT GCC TTC ACA TAG TCA TCC TTG CGC) plus 100 μmol of each dNTP , 3 μL of 10 X PCR buffer (100 mM Tris-HCl pH 9.0 25 °C, 500 mM KCl), 1.2 mM $MgCl_2$, 100 ng DNA and 1 unit of Taq DNA polymerase. Amplification was for one cycle of 2 min at 95 °C, 40 cycles of 0.5 min at 94 °C, 0.5 min at 48 °C and 0.5 min at 72 °C, and a final 10 min extension at 72 °C. The PCR products were digested with the *Bst*UI restriction enzyme and

the digestion products separated by 3% (w/v) agarose gel electrophoresis. The wild-type genotype gave bands at 144 bp and 26 bp, the heterozygous mutant genotype at 170 bp, 144 bp and 26 bp, and the homozygous mutant genotype at 170 bp.

Statistical Analyses

The chi-square (χ^2), Mann-Whitney U and Kruskal Wallis tests were used for univariate analysis. Bonferroni's correction was used to adjust p values for multiple comparisons between individuals with combined genotypes and their 1-OHP levels to identify the relationship according to the Kruskal Wallis test results. Univariate regression analysis was carried out with 1-OHP as the dependent variable. The independent variables for genotypes were defined as variants = 1 and wild type = 0. In the multivariate method, independent variables that turned out to be non-significant in the multiple regression analysis were excluded by a backward stepwise procedure. The stepwise likelihood ratio test was used to evaluate the difference between all groups by accepting the 1-OHP concentration as the dependent variable and age, smoking (y/n), duration of occupational exposure, CYP1A1m2, CYP1B1m2, GSTM1, GSTT1, GSTP1 (Ile105Ala) and GSTP1 (Ala114Val) genotypes as independent variables. All statistical procedures were performed with the SPSS statistical package version 10 (Chicago, IL/USA). P-values < 0.05 were considered significant.

Results

The Turkish coke oven workers sampled by us displayed a mean urinary 1-OHP concentration (1.68 ± 2.39 μmol mol^{-1} of creatinine) which was significantly higher ($p < 0.001$) than the mean (0.34 ± 0.42 μmol $mol^{-1}$of creatinine) shown by the controls (Table 2). In non-smokers, there was a significant difference ($p < 0.01$) in the mean urinary 1-OHP levels between coke oven workers (1.13 ± 1.15 μmol mol^{-1} of creatinine) and controls (0.28 ± 0.23 μmol mol^{-1} of creatinine). In smokers there was also a significant difference ($p < 0.001$) in the mean urinary 1-OHP levels between coke oven workers (1.99 ± 2.83 μmol mol^{-1} of creatinine) and controls (0.35 ± 0.44 μmol mol^{-1} of creatinine). Thus, the mean urinary 1-OHP levels of non-smoking coke oven workers was significantly higher ($p < 0.001$) than that of both smokers and non-smokers in the control group. In both, the coke oven workers group and the control group, there was no significant difference in the mean urinary 1-OHP levels of smokers and non-smokers.

There were no mutant homozygous CYP1A1*m2 Ile462Val* and GSTP1 *Ala114 Val* genotypes in either coke oven workers or controls. In addition, due to the small number of CYP1B1 and GSTP1 homozygous mutant genotypes in the coke oven workers (CYP1B1*m2 Ser/Ser* n = 2, GSTP1 *Val105Val* n = 2) and controls (CYP1B1*m2 Ser/Ser* n = 3, GSTP1 *Val105Val* n = 2) the homozygous mutant and heterozygous genotypes were combined for statistical analysis.

In coke oven workers, genetic polymorphisms of CYP1A1m2, CYP1B1m2, GSTM1, GSTT1, GSTP1 (Ile105Val) and GSTP1 (Ala 114Val) did not show any influence on 1-OHP excretion (Table 3). However, in the controls the mean urinary 1-OHP levels of individuals carrying the GSTT1-genotype was significantly higher ($p < 0.01$) than that of those carrying GSTT1+ genotype (Table 3).

Multivariate regression analysis was performed with urinary 1-OHP concentration as the dependent variable and age, occupational exposure, smoking (y/n), CYP1A1m2, CYP1B1m2 GSTM1, GSTT1, GSTP1 (Ile105Val) and GSTP1 (Ala 114Val) genotypes as the independent variables. The multivariate analysis showed that duration of occupational exposure and GSTT1 metabolic genotype were significant predictors (both at $p < 0.0001$) of urinary 1-OHP levels (Table 4). Multivariate analysis also indicated that the GSTM1 genotype showed no, or only a marginal, quantitative effect on urinary 1-OHP excretion ($p = 0.092$).

In addition, controls with the combined GSTM1-/GSTT1- genotype (1.22 ± 1.05, mean ± standard deviation (SD), n = 4) had significantly higher levels of urinary

Table 2 - Mean ± standard deviation (SD) concentration (μmol mol^{-1} creatinine) of urinary 1-hydroxypyrene (1-OHP) coke oven workers and controls. Mann-Whitney U-test Significant differences are also shown in the footnote.

	Number of individuals (n) and urinary 1-OHP concentration					
	Workers			Controls		
	n	Mean ± SD	Range	n	mean ± SD	Range
Overall	50	1.68 ± 2.39[a]	0.05 to 14.99	50	0.34 ± 0.42	0.01 to 2.69
Non-smokers	18	1.13 ± 1.15[b, c]	0.05 to 3.77	7	0.28 ± 0.23	0.12 to 0.79
Smokers	32	1.99 ± 2.83[d, e]	0.37 to 14.99	43	0.35 ± 0.44	0.01 to 2.69

[a]Significant at $p < 0.001$ compared to the controls. [b]Significant at $p < 0.001$ compared to control group smokers. [c]Significant at $p < 0.01$ compared to control group non-smokers. [d]Significant at $p < 0.001$ compared to control group non-smokers. [e]Significant at $p < 0.001$ compared to control group smokers.

Table 3 - Mean ± standard deviation (SD) concentration (μmol mol^{-1} creatinine) of urinary 1-hydroxypyrene (1-OHP) coke oven workers and controls with respect to metabolic genotypes.

	Number of individuals (n), urinary 1-OHP concentration and p value							
	Workers				Controls			
Gene and genotype	n	Mean ± SD	Range	p value	n	Mean ± SD	Range	p value
CYP1A1 exon7 Ile/Ile	44	1.70 ± 2.49	0.05 to 14.99	0.881	46	0.34 ± 0.43	0.01 to 2.69	0.411
CYP1A1 Ile462Val le/Val	6	1.50 ± 1.55	0.46 to 4.43		4	0.35 ± 0.19	0.15 to 0.52	
CYP1B1 exon7 Asn/Asn	25	2.23 ± 3.16	0.05 to 14.99	0.214	25	0.42 ± 0.55	0.01 to 2.69	0.367
CYP1B1 Asn453Ser Asn/Ser, Ser/Ser	25	1.12 ± 1.02	0.37 to 5.01		25	0.26 ± 0.20	0.05 to 0.79	
GSTM1 +/+, +/-	25	1.71 ± 2.90	0.05 to 14.99	0.877	26	0.25 ± 0.18	0.01 to 0.68	0.187
GSTM1 -/-	25	1.65 ± 1.81	0.23 to 7.63		24	0.45 ± 0.56	0.05 to 2.69	
GSTT1 +/+, +/-	43	1.40 ± 1.47	0.05 to 7.63	0.548	41	0.25 ± 0.19	0.01 to 0.79	0.013*
GSTT1 -/-	7	3.41 ± 5.24	0.38 to 14.99		9	0.74 ± 0.81	0.12 to 2.69	
GSTP1 exon5 Ile/Val	30	1.58 ± 2.66	0.25 to 14.99	0.751	32	0.39 ± 0.49	0.07 to 2.69	0.203
GSTP1 Ile105Val Ile/Val, Val/Val	20	1.82 ± 1.97	0.05 to 7.63		18	0.25 ± 0.22	0.01 to 0.74	
GSTP1 exon6 Ala/Ala	43	1.66 ± 2.48	0.23 to 14.99	0.922	44	0.37 ± 0.44	0.01 to 2.69	0.081
GSTP1 Ala114Val Ala/Val	7	1.79 ± 1.88	0.05 to 5.01		6	0.17 ± 0.17	0.05 to 0.51	

*GSTT1 null genotype is significantly different from GSTT1 positive genotype (Mann-Whitney U-test at $p < 0.05$).

1-OHP than controls with the GSTM1+/GSTT1+ (0.69 ± 2.15, n = 21, $p < 0.001$), GSTM1-/GSTT1+ (0.29 ± 0.22, n = 20, $p < 0.001$) or GSTM1+/GSTT1- (0.36 ± 0.24, n = 5, $p < 0.01$) genotypes.

Although the 1-OHP levels of control group non-smokers were not significantly different from those of control group smokers, 34 (1-OHP = 0.24 ± 0.19) out of 41 GSTT1+ individuals and all nine (1-OHP = 0.74 ± 0.81) GSTT1- individuals (Table 3) were smokers and the difference in 1-OHP levels between these two genotypes was significant at $p < 0.01$ when taking only the smokers into account. In addition, 17 out of 21 GSTM1+/GSTT1+ individuals (1-OHP = 0.23 ± 0.17), 17 out of 20 GSTM1-/GSTT1+ individuals (1-OHP = 0.27 ± 0.21), and all the GSTM1+ plus GSTT1- individuals (1-OHP = 0.36 ± 0.24) and GSTM1-/GSTT1- individuals (1-OHP = 1.22 ± 1.05) were smokers. The differences in mean urinary 1-OHP between the smokers with the GSTM1-/GSTT1- genotype and those with the GSTM1+/GSTT1+ genotype was significant ($p < 0.001$), the same being true for the GSTM1-/GSTT1+ ($p < 0.001$) and GSTM1+/GSTT1- ($p < 0.01$) genotypes as compared to the GSTM1-/GSTT1- genotype. The non-smokers may also have been exposed to PAHs by several other routes *e.g.* environmental tobacco smoke.

Table 4 - Multivariate regression analysis of the effects of several selected variables on urinary 1-hydroxypyrene (1-OHP) levels of coke oven workers and controls by a backward stepwise procedure.

Independent variable[a]	Exp B[b]	p	Lower	Upper
Occupational exposure	6.241	0.0001	3.757	10.369
GSTT1	2.671	0.001	1.479	4.824
GSTM1	1.423	0.092	0.944	2.147

[a]Variables described in Materials and Methods.
[b]Regression coefficient.

Discussion

We investigated whether the CYP1A1, CYP1B1, GSTM1, GSTP1 and GSTT1 polymorphisms have any influence on urinary 1-OHP levels of Turkish coke oven workers, possibly exposed to polycyclic aromatic hydrocarbons (PAHs), and controls from the same factory who should not have had occupational exposure to PAHs. To our knowledge, this is the first study performed in Turkey in regard to this subject.

In this study, biological monitoring of the internal PAH dose was done by analysis of urinary 1-OHP which has been suggested as a biomarker of PAH exposure because of the high sensitivity of the analytical method and because it takes into account all exposure routes (Burgaz *et al.*, 1998; Carstensen *et al.*, 1999; Alexandrie *et al.*, 2000). We found that the concentration of urinary 1-OHP of Turkish coke oven workers was significantly higher (about five-fold) than that of the controls. Similar results have been obtained in previous studies (Wu *et al.*, 1998; Zhang *et al.*, 2001; Hanaoka *et al.*, 2002). In Chinese coke oven workers the increase was up to 21.8-fold (Hanaoka *et al.*, 2002), although the mean 1-OHP value in Chinese coke oven workers reported by Hanaoka *et al.* (2002) was 10.9 μmol mol^{-1} of creatinine, or about 6.5 fold higher than the mean of 1.68 μmol mol^{-1} of creatinine found in our study. These findings clearly indicate that the exposure of PAH is rather

high in coke oven workers and/or that, in Chinese coke oven workers, the use of protective equipment and proper clothing is poor. The lower levels of 1-OHP found in our study were similar to the mean urinary 1-OHP levels of Swedish aluminum factory workers (Carstensen *et al.*, 1999; Alexandrie *et al.*, 2000), probably explainable by the continuous use of protective equipment and clothing by the workers in our study. However, in our study 16% of urinary 1-OHP levels in coke oven workers still exceeded the occupational exposure limit (OEL) of 2.3 μmol mol^{-1} of creatinine (Jongeneelen, 2001). These results indicate that up to 1/6 of the workers in the coke factory studied have high PAH absorption despite the use of protective equipment, suggesting that individual safety precautions may not be being used effectively by some of the workers studied. On the other hand, the concentration of urinary 1-OHP of control group in our study was lower than that of the Chinese controls (Hanaoka *et al.*, 2002; Leng *et al.*, 2004) but higher than Sweden, Italian and Korean controls (Carstensen *et al.*, 1999; Alexandrie *et al.*, 2000; Yang *et al.*, 2003). The discrepancies observed between the studies could be due to the distinct life style factors of the populations studied, such as smoking habits, passive smoking, diet and heating with coke at home.

In our study, there was an increase in urinary 1-OHP concentrations of the smokers in coke oven workers group as compared to those of the non-smokers in the same group, but this was not significant (Table 2). This is in agreement with other studies of coke oven workers (Zhang *et al.*, 2001), iron foundry workers (Carstensen *et al.*, 1999; Alexandrie *et al.*, 2000) and asphalt workers (Burgaz *et al.*, 1998). Similarly, we found that smoking had no significant effect the urinary 1-OHP levels of the control group. In this case, conflicting results have been reported, with some studies reporting that smoking had no effect on urinary 1-OHP levels (Tjoe *et al.*, 1993; Burgaz *et al.*, 1998; Nan *et al*., 2001; Zhang *et al.*, 2001) whereas other studies have reported a difference (Carstensen *et al.* 1999; Alexandrie *et al.* 2000; Nerurkar *et al.* 2000). It is interesting to note that in our study the urinary 1-OHP levels in the non-smoking coke oven workers were significantly higher than those of the smokers in the control group, indicating that the occupational exposure to PAHs was much higher than the exposure produced by smoking (Table 2).

Interindividual differences in the rate of metabolism (activation/detoxification) of PAHs have generally been thought to be due to enzyme polymorphisms (Carstensen *et al.*, 1999; Alexandrie *et al.*, 2000). By establishing a linkage between urinary 1-OHP levels and genetic polymorphism of specific genes it is theoretically possible to identify those individuals who might have an increased risk of developing cancer as a result of their exposure to PAHs (Apostoli *et al.*, 2003), with studies having focused on cytochromes as PAH activators and glutathione transferases as PAH detoxifiers (Alexandrie *et al.*, 2000; Apostoli *et al.*, 2003; Rihs *et al.*, 2005).

In our study we found no association between urinary 1-OHP excretion and CYP1A1, CYP1B1, GSTM1 or GSTP1 polymorphisms in either coke oven workers or controls, supporting other studies which have reported no association between these four enzymes and 1-OHP excretion in coke oven workers (Ovrebo *et al.*, 1998; Pan *et al.*, 1998; Nan *et al.*, 2001; Van Delft *et al.*, 2001; Apostali *et al.*, 2003; Rihs *et al.*, 2005) and controls (Yang *et al.*, 2003). On the other hand, a positive association has been reported between urinary 1-OHP excretion and CYP1A1, GSTM1, GSTP1 or GSTT1 polymorphisms in PAH exposed workers (Wu *et al.*, 1998; Alexendrie *et al.*, 2000; Schoket *et al.*, 2001; Zhang *et al.*, 2001).

In our study, the GSTT1 polymorphism by itself, or in combinations, showed no significant influence on urinary 1-OHP excretion in coke oven workers, supporting previous studies on coke oven workers (Alexandrie *et al.*, 2000; Van Delft *et al.*, 2001; Rihs *et al.*, 2005). However, to our knowledge, our study is the first to show a significant increase in the urinary 1-OHP of GSTT1-controls and, moreover, the trend was more pronounced in controls who were also GSTM1-, the mean urinary 1-OHP levels in these control individuals being almost as high as in the coke oven workers at 1.22 ± 1.05 μmol mol^{-1} of creatinine for the GSTM1-/GSTT1- control individuals as compared to 1.68 ± 2.39 μmol mol^{-1} of creatinine for the overall coke oven workers.

However, inconsistent results have been reported in the literature regarding the association between the GSTT1 genotypes and urinary 1-OHP excretion. Yang *et al.* (2003) found higher 1-OHP levels in GST1+ genotypes than the GSTT1- genotypes in smokers whereas other workers (Hong *et al.*, 1999; Alexandrie *et al.*, 2000; Nan *et al.*, 2001; Wu *et al.*, 2004b) could not find this in coke oven workers or smokers. It is known that 1-OHP is mainly excreted as the glucuronide conjugate. Although GSTM1 and GSTT1 have been reported to have a role in the elimination of epoxides and diol epoxides (Ketterer and Mulder, 1990), the role of GSTT1 in the removal of pyrene is still not clear and *in vitro* kinetic studies are needed to clarify it. Alexandrie *et al.* (2000) reported that GSTM1 or GSTT1 deficiency may increase the glucuronidation pathway as a result of the accumulation of PAH derivatives that are otherwise conjugated to glutathione. It is also known that deficiency in glutathione conjugation may also increase the induction of CYP1A1 and thereby increase formation of the 1-OHP glucuronide (Vaury *et al.*, 1995).

High exposure to PAHs generally affects the most sensitive genes, and an increase of PAH-DNA adducts in sensitive genotypes is to be expected. This direct effect has been shown in lung cancer patients with GSTM1- (Kihara *et al.*, 1994) and PAH-albumin adducts (Autrup *et al.*, 1995). These effects may be due to a more gene-environ-

ment interactions at high levels of exposure to PAHs when CYP1A1 will be induced and there may not be enough GST enzymes to detoxify PAH derivatives. However, a correlation between low smoking intensity and lung cancer or the CYP1A1 polymorphism has been shown and, moreover, a significant increase of the DNA adduct level in low exposure of sensitive individuals was observed, but there was no difference between sensitive and non-sensitive individuals in high exposure groups (Taioli *et al.*, 1995; Garte *et al.*, 1997). The saturation of enzymes may be a reason for this reverse relationship (Vineis and Martone, 1995; Garte *et al.*, 1997). This is supported by Merlo *et al.* (1998) who found a significant correlation between CYP1A1 Msp1 and 1-OHP excretion at low levels of exposure to PAHs and by Hemminki *et al.* (1997) who showed a significant increase of DNA-adducts at low levels of PAH exposure, similar findings having been reported by Georgiadis *et al.* (2001). The results for our low PAH exposure group, the controls, were in good agreement with the results reported by the aforementioned authors.

In conclusion, our results show that urinary 1-OHP is a sensitive indicator of recent human exposure to PAHs and that genetic polymorphism of GSTT1 may also to some extent reflect the interindividual variation in susceptibility to PAHs only at low PAH exposures.

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