

Urinary PAH Metabolites Influenced by Genetic Polymorphisms of *GSTM1* in Male Hospital Incinerator Workers

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Abstract: Urinary PAH Metabolites Influenced by Genetic Polymorphisms of *GSTM1* in male Hospital Incinerator Workers: Kyoung Ho LEE, *et al.* Department of Preventive Medicine, Seoul National University College of Medicine, Institute for Environmental Medicine, SNURC Korea—Hospital

waste incinerator workers are exposed to various pyrolysis products including polycyclic aromatic hydrocarbons (PAHs). We evaluated their exposure by assessing urinary 1-hydroxypyrene glucuronide (1-OHPG), as an internal dose of PAH exposure. The potential effect of genetic polymorphisms of *GSTM1/T1* involved in PAH metabolisms was also investigated. Pre- and post-shift samples were collected from 28 hospital incinerator workers. Urinary 1-OHPG was assayed by synchronous fluorescence spectroscopy (SFS) after immunoaffinity purification with the monoclonal antibody 8E11. Genotypes of *GSTM1/T1* were assessed by PCR-based methods. Information on smoking habits and use of personal protective equipment were collected by means of a self-administered questionnaire. The Mann-Whitney test was used to compare group means of these biomarkers. Urinary 1-OHPG levels were similar in pre- and post-shift urine samples. The arithmetic mean concentrations of urinary 1-OHPG were 0.16 ± 0.04 $\mu\text{mol/mol}$ creatinine pre-shift and 0.19 ± 0.09 $\mu\text{mol/mol}$ creatinine post-shift, but urinary 1-OHPG levels were significantly higher in individuals with the *GSTM1* null genotype than with the *GSTM1* present genotype ($p=0.05$, by Mann-Whitney test). Our results suggest that the urinary 1-OHPG levels in hospital waste

incinerator workers may be modified by the *GSTM1* genotype, but these findings remain to be confirmed in future studies involving larger sample sizes.

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Key words: Polycyclic aromatic hydrocarbon, Hospital incinerator workers, 1-hydroxypyrene glucuronide, Genetic polymorphism, Glutathione S-transferases

Hospital waste incineration has been the main method for disposing of a wide range of combustible materials, such as polyvinyl chloride plastics, papers and discarded items of equipment that constitute biomedical waste, because it can significantly reduce the volume of waste material and can also destroy organic matter¹. Materials with low heating values, such as full urine bags and dense body parts, may burn more slowly than the surrounding material and not be completely destroyed during incineration¹. Individuals working at incinerating plants, such as hospital waste incinerator workers, are exposed to various pyrolysis products including dioxin, carcinogenic heavy metals, and polycyclic aromatic hydrocarbons (PAHs)².

Urinary 1-hydroxypyrene (1-OHP) and a glucuronide conjugate of 1-OHP, 1-hydroxypyrene glucuronide (1-OHPG), have been successfully applied to individuals with a varying degree of PAH exposure as an internal dose of PAH exposure^{3, 5–8}. We evaluated their exposure by assessing urinary 1-OHPG.

Glutathione S-transferases (GSTs) catalyze the conjugation of glutathione to several electrophilic compounds, including carcinogenic PAHs⁴. And GSTs therefore play an important role in the detoxification of endogenous and exogenous toxicants⁹. GST class μ , encoded by the *GSTM1* ($\mu 1$) gene, catalyses the conjugation of many compounds, including epoxy

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derivates of PAHs¹⁰. *GSTM1* can detoxify PAHs, such as benzo[a]pyrene (BaP)¹¹. *GSTT1* is mainly involved in the metabolism of low molecular carcinogenic compounds (e. g., halomethane, dichloromethane, etc.)^{12, 13}. It is also involved in the formation of BaP-induced DNA-protein crosslinks in cultured human lymphocytes and increased levels of urinary 1-OHPG in smokers^{14, 15}. Individuals homozygous for the *GSTM1* and *T1* (theta 1) null alleles lack enzyme activity due to this deletion. About 50% of the Asian population have homozygotic deletion of the genes (*GSTM1/T1* null)¹⁶.

In this study, urinary 1-OHPG was assessed as an internal dose of PAH exposure in hospital incinerator workers. The potential effect of genetic polymorphisms of *GSTM1* and *T1* involved in PAH metabolisms were also investigated.

Material and Methods

1) Study subjects and sample collection

The study population consisted of 28 workers (average age 48.2, range 32–58) recruited from a hospital waste incinerating plant in South Korea. Each subject gave written informed consent and the study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital.

Pre- and post-shift samples were collected from 28 hospital incinerator workers. Pre-shift urine samples were collected before work and post-shift urine samples were collected after five days work. Pre- and post-shift urine samples were collected from the same workers. Urine samples (50 ml) were collected in polypropylene tubes for all the subjects during the sampling day and stored at –20°C until the analysis of creatinine and 1-OHPG. Blood samples (5 ml) were collected in EDTA sterile vacutainer tubes at the same time and encoded with identification numbers. Blood samples of three workers could not be collected. The samples were stored at –70°C until used for genotype determination. A self-administered questionnaire was used to obtain information about demographic characteristics, smoking habits, job history, alcohol consumption and use of personal protective equipment.

2) Urinary 1-hydroxypyrene glucuronide (1-OHPG) measurement

Urinary 1-OHPG levels were assayed with the assay developed by Strickland *et al.* (1994)⁵. Briefly, a four ml aliquot of each urine sample was treated with 1N HCl at 90°C for 1 h to hydrolyze acid-labile conjugated metabolites. 1-OHPG in urine was stable after acid hydrolysis (the recovery was 82%). And then loaded onto methanol/water primed Sep-Pak C18 cartridges (Waters, Milford, MA, USA). They were washed with the same volume of water and 30% methanol in water and eluted by the same volume of 80% methanol in water.

The elutes were concentrated to about 1/8 of the original volume under vacuum with mild heating, after which the volume was adjusted up to 4 ml with 7.5 mM phosphate-buffered saline (PBS) (pH 7.4). The samples were loaded onto immunoaffinity columns (IAC), which consist of CNBr-activated Sepharose 4B coupled with monoclonal antibody 8E11 that recognizes several PAH-DNA adducts and metabolites, including 1-OHPG, followed by washing with 4 ml of 7.5 mM PBS and 25% methanol in 7.5 mM PBS. 1-OHPG fractions were eluted with 70% methanol in 7.5 mM PBS and then dried. After re-dissolving in 2 ml of water, the samples were measured by synchronous fluorescence spectroscopy (SFS) (Perkin Elmer LS50B Luminescence spectrometer, Norwalk, CT, USA) with wavelength difference 34 nm excitation-emission. The recovery of the assay was 82% and the coefficient of variation of the assay was 9%.

3) Determination of genetic polymorphisms

The *GSTM1* and *GSTT1* genotypes were detected essentially as described elsewhere¹⁷. PCR incubations were carried out in: 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 0.2 mM of each deoxynucleotide triphosphate; β -globin specific primers were used with *GSTM1* or *GSTT1* specific primers in a multiplex PCR. After an initial denaturation at 94°C for 5 min, amplification were performed for 34 cycles with denaturation at 94°C for 20 s, annealing at 57°C for 20 s and extension at 72°C for 45 s. The process was completed in a final cycle at 72°C for 5 min. The PCR products were detected by electrophoresis on a 3% metaphor agarose gel (FMC BioProducts Cat. 50180). The results of duplicated analysis from other studies showed 94% almost identical outcomes.

4) Statistical methods

Differences between pre- and post-shift samples in urinary 1-OHPG were evaluated by Mann-Whitney test. It was also used to compare groups according to genetic polymorphisms. All statistical analyses were performed with SPSS statistical package version 10.0 (SPSS Inc., Chicago, IL, USA). The criterion for significance was set at $p < 0.05$.

Results

Urinary 1-hydroxypyrene glucuronide (1-OHPG) levels were similar in both pre- shift and post-shift urine samples ($p = 0.60$, by Mann-Whitney test, Table 1), but the arithmetic mean concentrations of urinary 1-OHPG were $0.16 \pm 0.04 \mu\text{mol/mol}$ creatinine pre-shift, lower than those post-shift ($0.19 \pm 0.09 \mu\text{mol/mol}$ creatinine) (Table 1). There was a statistically significant dose-responsive increase in urinary 1-OHPG levels with the increase in the number of cigarettes smoked per day (Pearson's correlation, $r = 0.48$, $p = 0.02$) (data not shown).

Table 1. 1-hydroxypyrene glucuronide levels ($\mu\text{mol/mol}$ creatinine) in relation to pre- and post-shift and *GSTM1* and *GSTT1* genotypes

Variables	N	A.M. ¹	S.D.	G.M. ²	Range	p-value*
Groups						
pre-shift	28	0.16	0.04	0.16	0.08–0.22	0.60
post-shift	28	0.19	0.09	0.17	0.09–0.33	
<i>GSTM1</i>						
null	10	0.21	0.06	0.20	0.16–0.33	0.05
present	15	0.16	0.07	0.14	0.08–0.32	
<i>GSTT1</i>						
null	7	0.20	0.07	0.19	0.12–0.32	0.30
present	18	0.17	0.07	0.16	0.08–0.33	

¹arithmetic mean, ²geometric mean, *by Mann-Whitney Test

Urinary 1-OHPG levels were significantly higher in individuals with the *GSTM1* null genotype (n=10, mean \pm SD=0.21 \pm 0.06) than with the *GSTM1* present genotype (n=15, 0.16 \pm 0.07) (p=0.05, by Mann-Whitney test) (Table 1).

Discussion

The ambient polycyclic aromatic hydrocarbons (PAHs) concentration was not assessed in hospital incineration workers. But exposure to most PAHs was through several pathways, such as thermal, inhalation and dietary exposure, and so on. Therefore, evaluation of the internal dose of PAHs, such as urinary 1-hydroxypyrene glucuronide (1-OHPG) and 1-hydroxypyrene (1-OHP) was important. Although urinary 1-OHP has been more widely applied for biomonitoring PAH exposure than 1-OHPG, the latter was measured in this study since it is a major pyrene metabolite in human urine, and it is a potentially a more sensitive biomarker for assessing low level exposure to pyrene in mixtures of PAHs^{5,6}.

Lee *et al.* found that urinary 1-OHPG levels were significantly higher in workers handling industrial wastes than in those with presumed lower exposure to PAHs¹⁸. But, we found that urinary 1-OHPG levels were not different in pre-shift (Monday morning) and post-shift (Friday evening) urine samples in hospital incineration workers. In a previous study, Jongeneelen *et al.* studied an operator who worked in a wood preserving plant and collected his urine daily to measure 1-OHP concentrations in two different periods: before and after work for 1 wk and during a 17 d period away from work¹⁹. They suggested that the excretion of 1-OHP is in two phases: a fast excreting component with a half-life of 1–2 d and a slow-excreting component with a half-life of 16 days. In our study, urinary 1-OHPG levels were not different in pre-shift and post-shift. This may be caused by a

carryover effect of the exposure in the previous week.

The role of GST enzymes in biotransformation of pyrene to 1-OHPG is not fully understood yet. Boyland and Sims (1964)²⁰ reported that the presence of the sulfate and glucuronic acid conjugates of pyrene metabolites in the urine and bile of both rats and rabbits treated with pyrene suggested the involvement of these phase II enzymes in the metabolism of pyrene. The *GSTM1* null genotype may stimulate the glucuronidation pathway by accumulating PAH derivatives that are otherwise conjugated to glutathione²¹. A previous study has shown an effect of the *GSTM1* genotype on urinary 1-OHPG levels in industrial incineration workers¹⁸. And in another study a small increase in urinary 1-OHP has been found among individuals with the *GSTM1* present genotype²². Urinary 1-OHP levels were also found to be higher in subjects with *GSTM1* present in a study of urban air exposure²³. In our study, a significant effect of the *GSTM1* null genotype on urinary 1-OHPG levels observed. The *GSTT1* genotype did not have any significant effect on urinary 1-OHPG levels, possibly due to the small sample size. Therefore, the likelihood of seeing an effect of *GSTM1* on PAHs detoxification would be increased by incineration emissions exposure.

In conclusion, our results suggest that the urinary 1-OHPG levels in hospital waste incinerator workers may be modified by the *GSTM1* genotype, but these findings remain to be confirmed in future studies involving larger sample sizes.

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