Exposure to Environmental Tobacco Smoke and Urinary 1-Hydroxypyrene Levels in Preschool Children

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Environmental tobacco smoke (ETS) contains relatively high concentrations of polycyclic aromatic hydrocarbons (PAHs). Urinary 1-hydroxypyrene (1-OHP), a metabolite of pyrene, is a good indicator of PAH exposure in occupational studies. In this study, we investigated the relationship between urinary 1-OHP concentration and ETS exposure in preschool children. Forty preschool children, aged 24–76 months, were studied during November and December, 1999. Two spot-urine specimens (one in the morning immediately after the subject woke up and the other at night before the subject went to bed) were collected 1 day after completion of a questionnaire, in order to determine 1-OHP concentrations by fluorescent spectrophotometry. Overall, urinary 1-OHP concentrations were relatively low but detectable (morning: median, 0.021 μg/g creatinine; range, 0.002–1.019 μg/g creatinine; night: median, 0.015 μg/g creatinine; range, 0.002–1.328 μg/g creatinine). Multiple linear regression analyses revealed that the total number of cigarettes smoked by the children's fathers during the 3 days prior to collection of the urine specimens was significantly associated with their urinary 1-OHP concentrations, after adjusting for other confounders. Each cigarette smoked by a child's father resulted in an average 9.6% increase in 1-OHP concentration in the morning urine specimen (95% confidence interval = 1.8–18.1%; $p = 0.02$). We did not find a significant increase in the 1-OHP concentration in night urine specimens ($p = 0.19$). Although the sample size was small, these findings indicate that urinary 1-OHP may be a suitable biomarker of ETS carcinogen exposure in children.

Key Words: environmental tobacco smoke, urinary 1-hydroxypyrene, preschool children

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Preschool children living in households with smoking parents are exposed involuntarily to environmental tobacco smoke (ETS) [1,2]. ETS contains approximately 4,000 chemicals, some of which are known human carcinogens, such as the polycyclic aromatic hydrocarbons (PAHs) benzo[*a*]pyrene, benzo[*a*]anthracene, dibenz[*ah*]anthracene, and 5-methylchrysene and other polyaromatic hydrocarbons [3,4]. PAHs contain four or five benzene ring compounds such as pyrene, benzo[*a*]pyrene, and benzo[*a*]anthracene and have been found in relatively high concentrations in cigarettes.

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These compounds are carcinogenic and may cause lung cancer in smokers [4]. Although benzo[*a*]pyrene is a more potent carcinogen than pyrene, it is transformed in the body into more than 20 metabolites [5]. The four-ring pyrene is almost exclusively metabolized to 1-OHP, which accounts for about 90% of the total urinary excretion of pyrene in all species studied to date, including humans [6,7]. Additionally, Ronchetti et al reported a high correlation between the urinary excretion of mutagens and urinary 1-OHP (Spearman correlation coefficient $r = 0.93$, $n = 12$) [2].

In several workplace environments, including coke-ovens, road-paving, and foundries, 1-OHP is a good indicator of exposure to PAHs [7–9]. Few studies have addressed the question of whether there is a significant relationship between exposure to PAHs and urinary 1-OHP in children [10–12]. In addition, information on ETS exposure in these studies was either not collected [10,11] or did not show any significant association [12]. Therefore, in this study, we investigated the relationship between exposure to ETS and urinary 1-OHP levels in children who had not started to attend primary school. The overall goal of this study was to find a suitable biomarker for epidemiologic studies of ETS-related cancer or ETS-associated intermediate markers of the carcinogenic process.

Materials and Methods

This cross-sectional study of preschool children was undertaken in one suburban county of southern Taiwan where there are no large industries. In total, 40 preschool children aged 24–76 months, volunteered by their parents, participated in this study. The study was approved by the Institute Review Board of the Harvard School of Public Health.

1-OHP in urine

Two spot-urine specimens from all 40 subjects were collected on a Sunday between November and December, 1999. The first sample was collected in the morning immediately after the subject awoke, and the second sample was taken at night before the subject went to bed. We instructed their parents (or grandparents) until they demonstrated they could correctly collect the child's urine specimens at home. The urine samples were stored at –20°C before analysis.

The 1-OHP concentration was determined using the method of Tolos et al [13]. Briefly, urinary 1-OHP concentrations were determined using high performance liquid chromatography (HPLC), fluorescence detection, and an HPLC manager for signal transformation. The HPLC run-time using isocratic elution (acetonitrile/water, 65/35 by volume) is 8 minutes and the retention time for 1-OHP is 5.2 minutes [14]. The calibration curve (6 points) was constructed using spiked urine samples (0.0–50.0 ng/ml) and the correlation coefficient was larger than 0.999. The recovery of the analyte was determined in spiked urine samples at two concentrations (3.50 and 10.67 ng/ml). The recovery, as mean ± standard deviation (coefficient of variation, CV), from three determinations was 110 ± 7 (6%) and 94 ± 4 (4%). The analysis of 1-OHP in urine was reproducible, as shown by repeated analysis of three samples (CV = 9%).

Creatinine in urine

Urinary creatinine was determined using a spectrophotometer (AU 5,000, Olympics, Kyoto, Japan) using the alkaline picrate method at Bioran Medical Laboratory (Cambridge, MA, USA). Urinary 1-OHP concentration was presented in units of μg/g creatinine.

Questionnaire on ETS exposure

A modification of the standard American Thoracic Society and the Harvard School Lung Health Survey questionnaire was used to obtain detailed information on cigarette smoking in the families of the 40 subjects [15,16]. The subject's parent (usually mother) filled out this questionnaire 1 day before the collection of urine specimens. There were detailed questions on smoking behavior in the home [17,18], both of parents and other household members. The estimated amount of daily smoking inside the home included the number of cigarettes smoked during the 24 hours and 3 days prior to urine collection by parents and non-parent smokers when the study child was in the same room [16]. The half-life of the initial phase of elimination in workers exposed to abundant PAH in the workplace ranges from 6 to 35 hours [10,11]. Therefore, timing of cigarette smoking exposure was crucial, since 1-OHP is more representative of recent ETS exposure. The questionnaire also contained questions on the study child: his/her frequency of intake of charbroiled food (e.g. food baked in wood-burning ovens, barbecued food) and exposure to wood or kerosene stoves.

Statistical analysis

Mean and standard deviation, as well as median and range were used to describe the distribution of age, Quetelet's index, cigarette smoking, and 1-OHP concentrations by exposure status. Spearman correlation coefficients (r) were used to study the relationship between urinary 1-OHP concentrations and ETS exposure. Multiple linear regressions were used to investigate the relationship between urinary 1-OHP and ETS exposure after adjusting for other confounders. Urinary 1-OHP concentrations were $\log_{10}$-transformed in order to normalize their distributions before multiple linear regression analyses. The estimated percent changes were calculated by exponentiating the regression coefficient by 10, subtracting one, and multiplying the results by 100% in multiple linear regression models. For example, the regression coefficient of $\log_{10}[1\text{-OHP}_{morning}]$ for subjects with ETS exposure from their father's smoking in the 24 hours prior to the collection of the urine specimen was estimated to be 0.08943 units increase in a crude analysis. Thus, the percent change for 1-OHP was estimated to be $[10^{0.08943} - 1] \times 100\% = 22.9\%$ (results shown in Table 2). Confidence intervals (CI) were similarly converted to percent changes. The SAS statistical package (SAS Institute Inc., Cary, NC, USA) was used and all p-values are two-sided [19].

Results

Demographic data and urinary 1-OHP concentrations are shown in Table 1. Twenty-two boys and 18 girls participated in this study; the median age was 57 months (range, 24–76 months). All study families used gas as their primary fuel in the house.

Table 1. Demographics and urinary 1-hydroxypyrene (1-OHP) concentrations among 40 preschool children

Demographic			Mean ± SD (min., med., max.)	*n* (%)
Age (months)			54.6 ± 14.6 (24, 57, 76)	
Quetelet's index (kg/m²)			15.3 ± 2.6 (10.5, 15.3, 21.6)	
Cigarette smoking (cigarettes)*				
Father	24 hours			
		At home	5.3 ± 7.9 (0.0, 0.0, 30.0)	
		In presence of subjects	2.2 ± 3.4 (0.0, 0.0, 15.0)	
	3 days			
		At home	22.1 ± 28.4 (0.0, 0.0, 90.0)	
		In presence of subjects	9.4 ± 12.9 (0.0, 0.0, 45.0)	
Others	24 hours			
		At home	4.9 ± 12.0 (0.0, 0.0, 70.0)	
		In presence of subjects	1.9 ± 5.0 (0.0, 0.0, 30.0)	
	3 days			
		At home	22.2 ± 43.6 (0.0, 0.0, 240.0)	
		In presence of subjects	8.4 ± 14.8 (0.0, 0.0, 75.0)	
1-OHP (μg/g creatinine)				
AM			0.069 ± 0.192 (0.002, 0.021, 1.019)	
PM			0.073 ± 0.221 (0.002, 0.015, 1.328)	
Gender				
Male				22 (55)
Female				18 (45)
Exposure to charcoal smoke at home				
24 hours				
Yes				2 (5)
No				38 (95)
3 days				
Yes				3 (7.5)
No				37 (92.5)

*Information on total exposure to environmental tobacco smoke or charcoal smoke at home was reported 24 hours and 3 days prior to urine specimen collection. Min. = minimum; med. = median; max. = maximum.

Fathers smoked in the range of 0–15 and 0–45 cigarettes in front of their children in the 24 hours and 3 days, respectively, prior to the collection of urine specimens (Table 1). No fathers smoked cigars and no mothers smoked either cigarettes or cigars (data not shown). In addition to fathers and mothers, other household members smoked in the range of 0–30 and 0–75 cigarettes in the presence of the children in the 24 hours and 3 days, respectively, prior to collection of urine specimens. No study children ate barbecued food in the 24 hours or 3 days prior to collection of urine specimens.

Overall, urinary 1-OHP concentrations were relatively low but detectable among the 40 preschool children (morning: median, 0.021 μg/g creatinine; range, 0.002–1.019 μg/g creatinine; evening: median, 0.015 μg/g creatinine; range, 0.002–1.328 μg/g creatinine). Urinary 1-OHP concentrations in the morning and at night were positively associated with the smoking status of the fathers in both crude and adjusted analyses (Table 2). The total number of cigarettes smoked by the children's fathers in the 3 days prior to collection of urine specimens was significantly associated with urinary 1-OHP concentrations on the subsequent morning ($p = 0.02$) after adjusting for age, gender, Quetelet's index, exposure to charcoal smoke (yes/no), and exposure to ETS from household members other than fathers. Each cigarette smoked by a child's father resulted in an average 9.6% increase in the morning urinary 1-OHP concentration (95%CI, 1.8–18.1%; $p = 0.02$). However, there was no statistically significant difference in the urinary 1-OHP concentration in specimens on the subsequent night ($p = 0.19$). We combined the number of cigarettes smoked by the study children's fathers and other household members and investigated their relationship with urinary 1-OHP concentrations in the children. Although more ETS exposure from fathers and other household members led to higher 1-OHP concentrations, they were not significantly different for the 24 hours (percent change, 3.8%; 95% CI, –8.1–17.2%; $p = 0.55$) or the 3 days prior to collection of urine specimens (percent change = 2.8%; 95% CI, –1.1–6.9%; $p = 0.17$) after adjusting for age, gender, Quetelet's index, and exposure to charcoal smoke.

Since most of the subjects' fathers did not smoke at home ($n = 23$), we excluded these children and analyzed the correlation between the average number of cigarettes smoked daily by the children's fathers in the 3 days prior to the collection of specimens and urinary 1-OHP concentrations. We found a high and significant correlation between urinary 1-OHP concentrations in the morning and at night ($r = 0.92$, $p < 0.0001$, $n = 17$). The average number of cigarettes smoked daily by the children's fathers in the 3 days prior to the collection of specimens was also positively correlated with urinary 1-OHP concentrations, especially those in the morning ($r = 0.49$ in the morning and 0.44 at night, $p = 0.045$ and 0.074, respectively, $n = 17$).

Discussion

In this study, the major predictor of morning urinary 1-OHP concentration in children was the average number of cigarettes smoked daily by their fathers in the 3 days prior to the collection of the urine specimen. An average increase of one cigarette smoked by the

Table 2. Relationship between urinary 1-hydroxypyrene (1-OHP) concentrations (μg/g creatinine) in preschool children and exposure to environmental tobacco smoke (ETS) from their fathers with and without adjusting for other confounders in multiple linear regression analyses ($n = 40$)

	Crude analyses		Adjusted analyses*	
	% change	95% CI	% change	95% CI
24 hours				
AM	22.9%	(–5.2–59.2%)	35.9%	(–2.2–88.8%)
PM	19.5%	(–10.4–59.3%)	14.8%	(–20.4–65.4%)
3 days				
AM	8.6%	(1.7–15.9%)	9.6%	(1.8–18.1%)
PM	8.7%	(–0.3–15.5%)	5.9%	(–2.7–15.2%)

*Adjusted for age, gender, Quetelet's index, exposure to charcoal smoke (yes/no), and exposure to ETS from household members other than fathers.

fathers resulted in an average 9.6% increase in 1-OHP concentration in the children's morning urine specimen. However, there was no statistically significant increase in the evening urine specimen ($p = 0.19$). Even though non-smoker fathers were excluded, the findings remain similar. These results suggest that childhood ETS exposure is mainly from subjects' fathers and urinary 1-OHP concentration can be used as a biomonitoring indicator of ETS exposure in children.

In epidemiologic studies, ETS has been demonstrated to cause acute and long-term adverse respiratory health effects in children [10,11]. Several studies have evaluated the association between lung cancer and passive smoke in adult women [20,21]. Both a longitudinal prospective study in Japan [20] and a case-control study in Greece [21] found a significant increase in the risk of lung cancer in nonsmoking women based on the amount of their husbands' smoking. ETS (sidestream smoke) contains higher levels of identified carcinogens than mainstream smoke directly inhaled by smokers [22]. One cigarette produces approximately 2.7–6.1 mg nicotine, 0.1 mg benzo [a]pyrene, and 0.5 mg pyrene [4]. Numerous studies have reported that urinary cotinine is a reliable measure of ETS exposure in children [23–25], but nicotine is not the principal carcinogen [26,27]. Therefore, urinary cotinine may not be an appropriate exposure biomonitoring indicator in epidemiologic studies of whether ETS is associated with carcinogenesis (e.g. PAH-DNA adducts).

Several studies have examined the effect of indoor or outdoor air pollution on urinary 1-OHP concentrations in children [10–12]. van Wijnen et al studied 644 Dutch children aged 1–6 years who were randomly selected from five different areas with various sources and levels of PAH in soil and ambient air [11]. They found that indoor sources of PAH, including open hearth, multi-burners, and smoking in the family, showed a small but significant positive association with urinary 1-OHP concentration in children (regression coefficient = 0.13, $p < 0.05$) after adjusting for other covariates. However, this study did not elucidate the relationship between smoking only in the family and urinary 1-OHP concentrations in children. Kanoh et al conducted an ecologic correlation study in 2,526 fourth- to sixth-grade children in Japan and found that schoolchildren living in highly PAH- and NO_x-polluted counties showed significantly higher urinary 1-OHP concentrations than those in less polluted counties [10]. Information on the smoking status of their parents was not collected in this study, so the investigators could not examine the effect of passive smoking on urinary 1-OHP concentrations in the children. In addition, these subjects may secretly smoke in school and interviewers may not have obtained the right information on their cigarette smoking status. Siwinska et al investigated the effect of indoor air pollution on 1-OHP concentrations in children who lived in the region of highest urbanization and industrialization in Poland [12]. In total, 412 children aged 7–8 years were recruited and their urine specimens were collected. The authors found that domestic heating and cooking with coal-burning stoves were the main contributors to increased 1-OHP concentrations. Although urinary 1-OHP concentrations tended to increase in children exposed to ETS, the result was not statistically significant ($p = 0.17$). In our study, 1-OHP concentrations in children were not significantly correlated with the smoking status of their father in the 24 hours prior to the collection of urine. However, we did find a significant association between urinary 1-OHP concentrations on the subsequent morning and the total number of cigarettes smoked by their father in the 3 days prior to the collection of urine specimens. In general, urinary 1-OHP concentration reflects recent exposure to PAH ($t_{1/2}$ = 6–35 hours) [8,10,11]. However, Jongeneelen et al collected the urine of an operator in a wood-preserving plant daily to measure 1-OHP concentrations in two different periods: before and after work for 1 week and during a 17-day period away from work [8]. They reported that the excretion of 1-OHP is biphasic: a fast-excreting component with a half-life of 1–2 days and a slow-excreting component with a half-life of 16 days. These results may partially explain why 1-OHP concentrations in children were significantly correlated with the smoking status of their fathers in the 3 days, rather than 24 hours, prior to urine collection.

In this study, none of the children's mothers smoked cigarettes and none of the children ate barbecued food in the 3 days prior to urine collection, so we could not evaluate their effect. Because few families used coal-burning stoves at home, we did not detect a significant effect from exposure to charcoal smoke on urinary 1-OHP levels in the children. We found that the number of cigarettes smoked by the father in the home was a major determinant of urinary 1-OHP concentration in children. These findings were not present for other

household members. The possible explanation for this finding is that information on cigarette smoking, determined by questionnaire, from other household members may not be as accurate as from the study children's fathers. This phenomenon will cause random misclassification and result in findings toward the null.

There were several limitations to this study. First, ETS exposure was measured by questionnaire, rather than other more accurate methods such as personal sampling. Second, we only evaluated 40 preschool children. Although the sample size was small, we still found an association between exposure to ETS from fathers and urinary 1-OHP concentrations on the subsequent morning in the children. Therefore, urinary 1-OHP concentration may be a feasible and appropriate biomarker of exposure to ETS in children.

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