

Urinary Cotinine and Exposure to Parental Smoking in a Population of Children with Asthma

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Background: Studies of the effects of tobacco smoke often rely on reported exposure to cigarette smoke, a measure that is subject to bias. We describe here the relationship between parental smoking exposure as assessed by urinary cotinine excretion and lung function in children with asthma.

Methods: We studied 90 children 4–14 years of age, who reported a confirmed diagnosis or symptoms of asthma. In each child, we assessed baseline pulmonary function (spirometry) and bronchial responsiveness to carbachol stimulation. Urinary cotinine was measured by HPLC with ultraviolet detection.

Results: Urinary cotinine concentrations in the children were significantly correlated ($P < 0.001$) with the number of cigarettes the parents, especially the mothers, smoked. Bronchial responsiveness to carbachol (but not spirometry test results) was correlated ($P < 0.03$) with urinary cotinine in the children.

Conclusion: Passive smoke exposure increases the bronchial responsiveness to carbachol in asthmatic children.

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Increased attention has been focused on the potential health effects of environmental tobacco smoke (ETS)⁶ on adults and children (1, 2). Increased symptoms such as chronic cough, chronic wheezing, and respiratory infections are observed in children exposed to parental smoking (3, 4). The long-term prevalence of these symptoms is unknown, hence new studies are required (5).

Studies on smoking and health have estimated exposure on the basis of the reported number of cigarettes

smoked daily (6), which is subject to bias and limitations in accuracy. Several biochemical tests, such as plasma or saliva thiocyanate, expired carbon monoxide, and carboxyhemoglobin (7), have been evaluated to measure cigarette smoke intake. However, they are not suitable because they lack sensitivity and specificity. Cotinine, one of the major metabolites of nicotine, provides more advantages because it is specific for exposure to nicotine from tobacco smoke, it is chemically stable, and urinary pH influences the excretion of cotinine less than it influences nicotine excretion (8). In addition, its longer half-life (19–40 h compared with 2 h for nicotine) (9–11) means that it reflects long-term exposure, whereas nicotine reflects recent exposure.

Numerous analytical techniques have been described to determine cotinine in biological specimens, including gas chromatography (12–14) gas chromatography–mass spectrometry (15), radioimmunoassay (16), enzyme-linked immunoassay (17), and HPLC (1–20). Here we used a simple, sensitive, and rapid HPLC method, suitable for processing a large number of samples.

This study was carried out to assess the effect of parental smoking on exposed and nonexposed children who had asthma. There are few studies that use urinary cotinine to measure documented passive exposure and its exacerbation of asthma.

Materials and Methods

SUBJECTS

A group of 90 children (54 boys and 36 girls), ages 4–14 years (mean, 8.04 years), was recruited from the pediatric asthma clinic, or among patients being followed up since admission to hospital with wheezing. The children were tested in the Respiratory Laboratory because of suspected asthma: 36% had chronic cough, 33% had clinical asthma,

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Received December 11, 1998; accepted December 30, 1998.

⁶ Nonstandard abbreviations: ETS, environmental tobacco smoke; SRaw, specific airway resistance; FEV₁, forced expiratory volume during 1 s; BR, bronchial responsiveness; and CCR, cotinine-to-creatinine ratio.

25% had recurrent wheezing bronchitis in the first 2 years of life, and 6% had isolated dyspnea or cough at exercise.

A trained interviewer administered a complete questionnaire to the accompanying parents about each child's illness and factors such as the presence of dogs, cats, or other animals in the house, carpet, rugs, and plush toys, all of which might cause sensitization. The parents were asked whether they were smokers, and how many cigarettes, cigars, or pipes of tobacco they smoked, inside and outside the house. One cigar or one pipe was recorded as equivalent to five cigarettes (21). Analysis of cotinine (see below) was blind to questionnaire results.

Approval was obtained from the Hospital Ethics Committee, and parents gave their written informed consent.

Most of the children were admitted on the same day in the week (Wednesday). The urine samples were collected after admission, early in the morning, in sterile bottles, and stored frozen (-20°C) until analysis.

COTININE ASSAY

Urinary cotinine was quantified by reversed-phase ion-paired HPLC with ultraviolet detection at 260 nm as described earlier (22). Briefly, 2-phenylimidazole, the internal standard, was first added to 10 mL of urine (or to 20 mL to increase sensitivity), and the pH was adjusted to 11 with 5 mol/L sodium hydroxide. The extraction was done on a solid phase Extrelut[®]-20 column (Merck), and the compounds were eluted with chloroform into a glass tube containing 100 μL of glacial acetic acid. The eluate was evaporated at 40°C under a nitrogen stream. The residue was dissolved with 1000 μL (or 100 μL to increase sensitivity) of mobile phase, and 20 μL was injected into the chromatograph for analysis.

The mobile phase was a mixture of 90 mL/L acetonitrile–30 mL/L methanol–880 mL/L buffer containing 0.3 mmol/L sodium octane sulfonate as an ion pairing reagent. The pH of the mobile phase was adjusted to 4.8 with triethylamine. This method presents some advantages because of a single-step solid-phase extraction on Extrelut, which simplified the extraction procedure considerably, thus reducing the analysis time and improving compatibility with routine analysis. The time required for an assay was 1.5–2 h. For serial assays, several extractions could be performed in parallel, which reduced to chromatographic time for one assay to 20 min.

Creatinine concentrations were determined using the Jaffé reaction on the DAX apparatus (Bayer Diagnostic).

CLINICAL TESTS

Spirometric tests and bronchial challenges were performed in the Respiratory Laboratory. Prior to carbachol challenge, each child performed baseline pulmonary function tests. The pulmonary function tests consisted of specific airway resistance (S_{Raw}) measurements with a constant body plethysmograph (model Master Lab; Jaeger), using the method of Dubois et al. (23). The children were instructed to breathe at a frequency of 2 cycles per

second, and the thoracic gas volume measured simultaneously was close to the functional capacity. The mean of five reproducible measurements was used each time. The forced expiratory volume during 1 s (FEV₁) was determined. The functional measurements were expressed as percentages of reference values reported by Zapletal et al. (24) corrected for age, sex, and height.

For carbachol inhalation, a standardized dosimeter technique was used: carbachol puffs were delivered by a dosimeter (ME-FAR dosimeter; Elletromedicalli), with a nebulization time of 1.2 s and a pause time of 5 s between two puffs. A carbachol solution (bronchoconstrictor) of 2 g/L was used, and 3 mL of the solution was placed in the nebulizer. While wearing a nose clip, the children were instructed to breathe quietly through a spacer device. Cumulated doses of carbachol were then administered. The S_{Raw} was measured 2–3 min after each inhaled dose of carbachol. The doubling dose (concentration of carbachol that produced a twofold increase in S_{Raw}, defined as sensitivity) was noted for each child. The lower the value of the doubling dose, the higher the bronchial responsiveness (BR) to carbachol.

Bronchodilators and cromoglycate were withheld for at least 12 h before tests. Every child was subjected to the carbachol challenge test, even those with FEV₁ <80% of the predicted value.

STATISTICAL ANALYSIS

Because the data did not follow a gaussian distribution, we used the Kruskal–Wallis one-way analysis of variance to test the differences between quantitative variables, and the nonparametric Spearman correlation coefficient to measure association. $P < 0.05$ was considered statistically significance. Statistical analysis was performed with Statistical Package for Social Sciences programs (SPSS Inc.).

Results

The children were divided into three groups: (a) not exposed to tobacco smoke at home, i.e., the parents did not smoke (39%); (b) slightly exposed, i.e., the parents smoked ≤ 10 cigarettes/day (23%); and (c) highly exposed, i.e., the parents smoked > 10 cigarettes/day (38%). The cigarettes per day cutoffs are based on total parental smoking, inside and outside the home.

Table 1 shows the median concentrations and interquartile ranges of the distribution of urinary cotinine and the cotinine-to-creatinine ratio (CCR), according to parental smoking state. The Kruskal–Wallis one-way analysis of variance was used to test differences between the three groups. The effect of parental smoking on cotinine concentration and on CCR was highly significant ($P < 0.000\ 001$).

A classification of children as passively exposed to smoking from only fathers, only mothers, and both parents was also established. Table 2 shows the medians of urinary cotinine concentration in the three groups: (a) only father smokes (48%), (b) only mother smokes (23%),

Table 1. Urinary cotinine according to self-reported^{a,b} parental smoking exposure.

Reported cigarettes per day	n	Urinary cotinine, $\mu\text{g/L}$		CCR, $\mu\text{g/g}$	
		Median	Interquartile range	Median	Interquartile range
None	31	0.000	0.000–0.000	0.000	0.000–0.000
1–10	18	1.000	0.000–8.000	0.500	0.000–9.000
>10	30	9.750	4.000–36.000	11.250	3.000–37.000
Total	79				

^a Number of values less than limit of determination (LOD = 0.5 $\mu\text{g/L}$): six for the group with no exposure; four for the group exposed to 1–10 cigarettes/day; and one for the group exposed to >10 cigarettes/day.

^b $P < 0.000\ 001$, Kruskal–Wallis.

and (c) both parents smoke (29%). We estimated cotinine excretion per cigarette (ratio of urinary cotinine concentration to total number of cigarettes the parents smoked per day) in the three exposure groups.

With the one-way analysis of variance, there was no significant difference between these three groups; however, the median concentration of cotinine was higher when only the mother smoked (20 $\mu\text{g/L}$) than when only the father smoked (3.5 $\mu\text{g/L}$); likewise, cotinine per cigarette was higher when only the mother smoked (1.8 $\mu\text{g/cigarette}$) than when only the father smoked (0.2 $\mu\text{g/cigarette}$).

To evaluate the effects of parental smoking on severity of wheezing and on bronchial response, we studied the associations between urinary cotinine concentration and both the response to the spirometric basal tests and the BR to carbachol administration. There was no apparent correlation between urinary cotinine and basal spirometric tests, but the correlation between cotinine and BR to carbachol was significant ($P = 0.03$). The same study with the CCR was not significant ($P = 0.07$). There was no significant correlation between the number of cigarettes the parents smoked and the BR to carbachol ($P = 0.19$).

Discussion

Passive inhalation by children has long been implicated as a definite threat in respiratory illness (25, 26). It is now

agreed that cotinine is the marker of choice for quantifying passive exposure to smoke. Nevertheless, most of the studies on risk factors for asthma and wheezing illness in childhood are performed using the number of cigarettes the parents smoke (27–29). However, children may be exposed to ETS from sources other than parental smoking. Background exposure is difficult to quantify (30, 31). In this study, we combined questionnaire responses and urinary cotinine assays to investigate the effects of exposure to ETS on asthmatic children.

Our results show that urinary cotinine concentration and CCR in children are highly correlated with the number of cigarettes the parents smoke. The difference of means between the three groups, nonexposed, slightly exposed, and highly exposed, was highly significant ($P < 0.001$). In this study, there is apparently no exposure to ETS among the 30 children of nonsmoking parents. This would seem to imply that children are exposed primarily through parental smoking. This point must be investigated in additional studies.

In agreement with previous studies (32–34), if only the mother smoked, children had higher urinary cotinine (20 $\mu\text{g/L}$) than if only the father smoked (3.5 $\mu\text{g/L}$). This is probably because young children spend comparatively more time with their mother, and the fathers smoke fewer of their daily cigarettes at home than do mothers. However, cotinine excretion for each cigarette per day (ratio of urinary cotinine concentration/total cigarettes smoked per day) did not differ significantly among these groups.

In our study, parental smoking had no apparent effect on the baseline pulmonary function tests. We found no significant relationship between urinary cotinine (or CCR) and either SRaw, which measures the bronchial diameter, or FEV₁ (maximal expiratory air volume). However, our study has some limitations that should be noted. We studied all the children, even those with a FEV₁ <80% ($n = 5$) of predicted value. They were not excluded because they represent children with the highest probability of having asthma or asthmatic symptoms. However, these results could be influenced by recent bronchodilator medications or by respiratory infections.

A strong positive association was found regarding passive smoking and BR to carbachol stimulation. Children of parents who smoked had lower bronchial sensi-

Table 2. Parental (daily) cigarette consumption, children's urinary cotinine, and their ratio.

	Cigarettes/day, mean	Urinary cotinine, $\mu\text{g/L}$		
		n	Median ^a	Interquartile range
Only father smokes	12.6	23	3.5	0–15
Only mother smokes	12.0	11	20	0–90
Both parents smoke	18.5	14	7.5	6–87
Cotinine per cigarette smoked each day, μg				
		n	Median ^b	Interquartile range
Only father smokes	12.6	23	0.2	0–0.8
Only mother smokes	12.0	11	1.8	0–4.7
Both parents smoke	18.5	14	0.36	0.14–1.1

^a $P = 0.22$, Kruskal–Wallis.

^b $P = 0.37$, Kruskal–Wallis.

tivity (e.g., higher BR), because the doubling dose of carbachol was significantly correlated ($P = 0.03$) with urinary cotinine concentration. This correlation is negative because the higher the cotinine concentration, the lower the bronchial sensitivity. This inverse correlation was also observed with CCR with a borderline probability of $P = 0.07$. Significance was not reached, however, when we measured the association between the BR and the number of cigarettes the parents smoked ($P = 0.19$). Previous studies found a significant difference with some tests but not with others (35–38). However, they did not evaluate smoke exposure with urinary cotinine but rather with the number of cigarettes the parents smoked, or only the mother smoked, which can introduce bias.

In conclusion, our results confirm that a dose–response relationship can be established between the intensity of passive exposure to tobacco smoke and urinary excretion of cotinine. Urinary cotinine may better reflect tobacco-smoke exposure than the questionnaire data on the number of cigarettes the parents smoked because questionnaires are subject to reporting bias. The effect of passive smoking on asthmatic children is evident on BR to carbachol stimulation, which is increased. Urinary cotinine, easy to quantify by our HPLC method (22), is probably the best biochemical marker of exposure to tobacco smoke, and it could be used in any respiratory and lung function epidemiological survey. Furthermore, knowing the urinary cotinine concentrations of their children could add incentive for parents to stop smoking.

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