The urinary cotinine levels of infants and the determinants

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The aim of this study was to determine the effect of the route of tobacco smoke exposure on urinary cotinine levels of infants.

A cross-sectional analysis was done on 254 six-month-old infants. The infants were grouped according to the route of tobacco smoke exposure. The urinary cotinine/creatinine ratios were determined.

Forty-nine percent (124/254) of mothers were smokers. Urinary cotinine levels in infants of smoking mothers were statistically significantly higher than levels in infants of non-smoking mothers. The highest mean cotinine/creatinine level was found in the breast-milk-exposed group. Linear regression analysis showed that maternal smoking increased urinary cotinine level by 541 times and breastfeeding increased it by 171 times, whereas early start of formula feeding decreased it by 63 times.

Tobacco exposure by breastfeeding may be more harmful than other routes of exposure. Mothers should be encouraged to stop smoking during the breastfeeding period even if they avoid exposing their infants to passive tobacco smoke.

Key words: Breastfeeding, cotinine, infant, tobacco smoke pollution.

Tobacco smoke is the most common toxin found in our environment. The frequency of smokers is around 44% in Turkey, and tobacco consumption in Turkey has increased by 22% over the last 30 years. Twenty-eight percent of Turkish women aged 15-49 years are smokers[1,2]. Such a high rate of smoking and increasing tobacco use can lead to an increasing incidence of tobacco-related diseases. Our children are exposed daily to increasing amounts of tobacco smoke. Passive tobacco smoking is considered among the most serious and most common health hazards for our children. Tobacco smoke exposure is known to lead to increased frequency of infections and growth retardation in infants and children. Furthermore, passive smoking has been observed at a high rate, and cigarette smoke is reported to be the most important factor increasing the symptoms of asthma[3]. Tobacco smoke has been shown to have negative effects on an infant's health, especially during the first year of life. However, it is questionable whether breastfeeding has any protective effect against the health risks caused by tobacco smoke exposure in infants. The aim of our study was to investigate the effect of the route of tobacco smoke exposure by measuring urinary cotinine levels in infants.

Material and Methods

This study was performed in 2005 as a cross-sectional analysis on 254 six-month-old infants who were brought to the healthy child outpatient clinic of Dr. Sami Ulus Children's Hospital. Hacettepe University Faculty of Medicine Ethics Committee approved the study (Date: 2.5.2002, No: FON02/12-12). The study protocol conformed to the provisions of the Declaration of Helsinki.

Informed consent was obtained from all mothers participating in the study. The infants whose

mothers accepted the conditions of the study entered the project. The mothers belonged to low-to-middle socioeconomic class.

The mothers were asked to complete a questionnaire, which included information about the education and occupation of both parents, birth season, the number of babies in the family, income of the family, health status of the mother, breastfeeding status of the infants, smoking status of mother, father and other family members, type of family, presence or not of another child at home less than five years of age, sex of infant, birthweight of infant, start of formula feeding, and total number of cigarettes smoked at home per day.

In our study, a mother smoking at least one cigarette per day was defined as a smoking mother. Smoking anywhere within the house was evaluated as exposure. Smoking outside the house was not considered as exposure to passive smoke. A nursing mother who smoked outside the house led to tobacco smoke exposure by breastfeeding only.

Infants were divided into 4 groups:

Group 1: Patients free from any exposure.

Group 2: Those who were exposed to cigarettes through breastfeeding (total number of cigarettes smoked in their homes varied between 15 and 40 per day).

Group 3: Those exposed passively (total number of cigarettes smoked in their homes varied between 15 and 40 per day).

Group 4: Those exposed both by breastfeeding and passively (total number of cigarettes smoked in their homes varied between 15 and 40 per day).

To determine the amount of tobacco smoke exposure of the infant, cotinine level in urine was measured in each patient. Urine samples taken from the infants for cotinine measurements were kept at -80°C until analysis. Cotinine measurement was performed by using gas chromatography-mass spectroscopy method (GC-MS-MS). A Model 6890 Agilent gas chromatograph equipped with a nitrogen phosphorus detector and a 10 m x.53 mm cross-linked used silica capillary column inlet system were used. Interassay variability was less than 7%.

Urinary creatinine concentrations were determined by the Jaffe rate method, using reagents supplied by Beckman, on an Astra routine analyzer. Cotinine level was obtained as cotinine/creatinine ratio (ng/mg).

For statistical analysis, significance was taken as $p<0.05$. Student's t-test for independent samples was used for the comparison of the means of two groups; chi-square test was used for comparison of n x 2 tables; and variance analysis was used for comparison of means of more than two groups. Linear regression analysis was done in order to determine independent factors affecting the urinary cotitine/creatinine ratio of infants. Forward likelihood ratio was chosen as a method. For entrance into the model, p was taken as 0.05, and for exclusion from the model, p was accepted as 0.10. The dependent factor was the urinary cotitine/creatinine ratio of infants. Independent factors were education and occupation of the mother and father, birth season, the number of babies in the family, income of the family, health status of the mother, breastfeeding status of the infants, smoking status of the mother, father and other family members, type of family, presence or not of another child at home less than five years of age, sex of infant, birthweight of infant, start of formula feeding, and total number of cigarettes smoked at home per day.

Results

Demographic characteristics of mothers are shown in Table I.

The study population consisted of 130 (51%) males and 124 (49%) female infants. One hundred twenty-one (48%) infants were breastfed alone, 12 (5%) were both on breastfeeding and supplementary feeding, 81 (32%) were brestfed for a period shorter than six months and then with formula, and 40 (16%) were fed with formula only. The comparisons of beginning of formula feeding, mean breastfeeding durations and mean birthweights of infants of smoking and non-smoking mothers are shown in Table II.

Forty-nine percent (124/254) of mothers were smokers. Fifty-eight percent (146/254) of fathers were smoking within the house. In 8% (21/254) of families, there was another smoker in addition to the mother and father.

Table I. Demographic Characteristics of Mothers (n=254)

Age of mother 29 ± 4.0 (23-28) years	
Education of mother	Total number of cigarettes smoked/day by mother
Illiterate 18 (7%)	0 130 (51%)
Primary 101 (40%)	One 4 (2%)
Secondary 78 (31%)	Five 52 (20%)
High school 40 (16%)	Ten 41 (16%)
University 17 (6%)	Twenty 27 (11%)
Occupation of mother	Smoking mothers resumed smoking after delivery in
Housewife 74 (29%)	One month 35 (28%)
Worker 77 (30%)	2 months 56 (45%)
Government official 63 (25%)	3 months 33 (27%)
Trades person 24 (9%)	
Employer 16 (7%)	

In 65% (165/254) of families, tobacco smoking took place within the house. Thirty-five percent (89/254) of the infants were not exposed to tobacco smoke at all, whereas 6% (15/254) were exposed only via breast-milk, 46% (117/254) only via passive smoking, and 13% (33/254) via breast-milk in addition to passive smoking.

Urinary cotinine/creatinine ratios of infants ranged between 0 and 2147.41 ng/mg (mean: 215.16±121.89 ng/mg). Urinary cotinine levels in infants of smoking mothers were statistically significantly higher than levels in infants of non-smoking mothers (429.5±524.7 ng/mg vs. 10.7±17.5 ng/mg, t=9.1, p<0.001).

Tobacco smoke exposure groups led to statistically significantly different mean urinary cotinine/creatinine levels (p<0.001) (Table III). Upon coupled comparisons of groups using the Student's t-test, the lowest average urinary cotinine/creatinine level was detected in the non-exposed group, and the highest mean cotinine/creatinine level was found in the breast-milk-exposed group. All groups were significantly different from each other (p<0.001).

Linear regression analysis was performed to determine independent factors affecting urinary cotitine/creatinine ratio of infants, and revealed the following effective factors (in decreasing order of significance): smoking status of mother, smoking status of father, smoking status of other familiy members, total number of cigarettes smoked at home, breastfeeding status of the infant, and start of formula feeding (p<0.05) (Table IV). Maternal smoking increased urinary cotinine level by 541 times, whereas father's smoking increased it by 2.4 times, other household member's smoking by 1.13 times, total number of cigarettes smoked at home per day by 4.2 units, and breastfeeding by 171 times; early start of formula feeding decreased urinary cotinine level by 63 times. Smoking status of the mother, father and other family members, total number of cigarettes smoked at home and breastfeeding status of infants increased urinary cotitine/creatinine ratio of infants, whereas early start of formula feeding decreased the ratio.

The reliability of self reports by the mothers related to household smoking was analyzed in Table V by using urinary cotinine levels of infants. Cut-off values for urinary cotinine were taken as <10 and ≥10. The sensitivity of maternal self report was found as 100% and specificity as 88.8%.

Discussion

According to the World Health Organization data, there are approximately 250 million women worldwide who are regular smokers. Only 3% of smoking women can succeed in quitting each year. Tobacco dependence is comparable to heroin dependence. Giving up smoking is not easy. Many women, especially the young ones who quit smoking during pregnancy, resume smoking within six months in the postpartum period. Although the prevalence of women who quit smoking during pregnancy has increased, more than half of the women who are smokers at the onset of their pregnancies continue to smoke since smoking is used as a stress-relieving mechanism by many new mothers[4]. In our study, all smoking mothers who quit smoking

Table II. Comparisons of Beginning of Formula Feeding, Mean Breastfeeding Durations and Mean Birthweights of Infants of Smoking and Non-Smoking Mothers (Mean±SD) (n=254)

		Smoker		Statistics
		Yes	No	
Only breastfeeding	No (n=121)	41 (34)	80 (66)	Chi-square test, χ^2= 40.6, p<0.001
Beginning of formula feeding	At birth (n=40)	16 (40)	24 (60)	
	2nd month (n=12)	7 (58)	5 (42)	
	3rd month (n=14)*	12 (86)	2 (14)	
	4th month (n=36)*	31 (86)	5 (14)	
	5th month (n=31)*	17 (55)	14 (45)	
Mean breastfeeding duration (month)		3.53±2.49	4.31±2.41	t=2.74, p=0.007
Birthweight		3356 ± 427.1	3560 ± 204.5	t= 4.88, p<0.001

(Numbers in parenthesis are percentages) (*indicates the group causing statistical significance)

during pregnancy resumed smoking within three months after delivery.

According to the 2003 Demographic and Health Survey of Turkey, the rate of exclusive breastfeeding at six months of age was only 1.8%; however, this rate was found to be 48% in our study population[1]. Nonsmoking mothers breastfed their infants significantly longer than smoking mothers. Additionally, smoking mothers started formula feeding significantly earlier than non-smoking mothers. Our results were compatible with the results of a study by Horta et al.[5] They showed that the probability of exclusive breastfeeding shorter than six months was twice as high for mothers smoking 20 or more cigarettes per day compared to non-smoking mothers. The same authors reported in a meta-analysis that mothers who resumed smoking before three months' postpartum started formula feeding 1.93 times more frequently than non-smokers[6].

The effect of maternal smoking on breastfeeding is still enigmatic. Early weaning in smoking mothers may be related to both physiological and psychosocial problems. Nicotine in the mother's bloodstream may reduce prolactin. Reduced prolactin inhibits the let-down reflex, which may cause a decrease in milk supply[7]. However, this hypothesis was not proven by measuring serum prolactin levels in smoking mothers. On the other hand, women who smoke seem to have significantly less motivation to breastfeed. Furthermore, the health care community may not support breastfeeding of smoking mothers because of their belief that nicotine-containing mother's milk would be harmful to the infant.

Urinary cotinine in infants may come from

Table III. Comparison of Mean Cotinine/Creatinine Ratios (ng/mg) of Tobacco Smoke Exposure Groups (mean±standard deviation)

Tobacco smoke exposure groups	Cotinine/Creatinine ratio (ng/mg)
None (n=89)	1.14 ± 0.56
Breastfeeding (n=15)	1461.1 ± 505.26
Passive (n=117)	108.93 ± 63.70
Breastfeeding + passive (n=33)	602.66 ± 244.37
Variance analysis	F=246, p<0.001

Table IV. Linear Regression Analysis of Independent Factors Affecting Urinary Cotitine/ Creatinine Ratio of Infants

	B	Beta	T	p	95% confidence interval	
Smoking status of mother	540.98	.603	5.7	.000	354.4	727.5
Smoking status of father	2.368	0.518	9.5	.000	1.220	3.517
Smoking status of other family members	1.13	0.166	3.3	.001	0.185	2.066
Total number of cigarettes smoked at home	4.2	0.191	3.1	.003	1.5	6.9
Breastfeeding	170.7	0.148	3.2	.002	5.7	284.2
Start of formula feeding (months)	-63.3	-.286	-4.4	.000	-91.4	-35.2

B value shows how many units the dependent variable changes when independent variable changes 1 unit.

three sources: nicotine inhaled by passive smoking; nicotine ingested with breast-milk; and maternal cotinine ingested with breast-milk and excreted by the infant unchanged. In our study, upon coupled comparisons of groups, the highest mean cotinine/creatinine level was found in the breast-milk-exposed group. Data from our investigation indicate that for infants of smoking mothers, breastfeeding rather than environmental tobacco smoke exposure by direct inhalation is one of the significant determinants of urine cotinine levels of infants. This difference can not be attributed to heavier smoking by breastfeeding mothers, since the number of cigarettes smoked per day was no different between groups.

According to our multivariate analysis results, smoking by the mother, father and any other household member, total number of cigarettes smoked at home per day and breastfeeding significantly increased urinary cotinine levels, whereas early start of formula feeding significantly decreased it independently. Maternal smoking and breastfeeding appear to be the strongest determinants of urinary cotinine level. We demonstrated that tobacco smoke exposure via breast-milk led to 13.4 times more nicotine exposure to the baby compared to passive exposure. Another finding of our study was that when the smoking mother starts formula feeding, urinary cotinine levels decreased by 63 times. Supporting our findings, Woodward et al.[8] demonstrated in their multivariate analysis that breastfeeding was the principal independent determinant of cotinine in infant's urine. The limitation of this study was that they compared only urinary cotinine level, not the urinary cotinine/creatinine ratio, which is a more reliable data in spot urine samples. Luck et al.[9] demonstrated that the significant serum concentrations and urinary excretion rates of nicotine in the breastfed infants of smoking mothers suggested that nursing contributed to the nicotine exposure of these neonates. Becker et al.[10] directly measured breast-milk cotinine and found a mean concentration as high as 495 ng/mg of creatinine, with a range of 347-707.

Mascola et al.[11] found urinary cotinine levels 2-to-8-fold higher in all of their smoking exposure categories than in previous reports. They explained this situation by the difference in the sensitivity of methods used to detect urinary cotinine levels, failure to adjust for urine creatinine levels and difference in the smoking rates of different communities. In addition, the

Table V. Reliability of Self Reports by the Mothers Related to Household Smoking Compared to Urinary Cotinine Levels of Infants

		Urinary cotinine/creatinine level ≥10	<10	Total
Household smoke	Yes	156	11	167
	No	0	87	87
Total		156	98	254

average age of their patient group was about 6-8 weeks, in contrast to the homogeneous age group of our study. We examined six-month-old infants, and our average urinary cotinine levels were comparable to other studies. Furthermore, we used a very sensitive method to measure urinary cotinine.

The major concern about the presence of nicotine in mother's milk is whether or not gastrointestinal absorption of this toxin is hazardous to infants. In adults, gastrointestinal absorption of nicotine is reduced possibly because of first-pass elimination effect of the liver. However, in infants, this first-pass effect and absorption could differ from adults. In this stage of life, there is an immaturity in first-pass elimination[12]. In the case of infants, age may be important. Cotinine elimination half-life in neonates is two to three times that in adults, and it is not known whether it is days, weeks or even months before infants develop the capacity to metabolize nicotine fully.

Some studies have shown that inhaled nicotine is more hazardous than ingested nicotine[12]. However, acute nicotine intoxication due to ingested nicotine has been reported[9]. On the other hand, cotinine is pharmacologically inactive, and it is unlikely that cotinine in breast-milk has any adverse effects on an infant's health. It is only a quantitative indicator of intake of other harmful metabolites[13]. Further investigations are needed of other harmful toxic substances in mother's milk, including their concentrations and their toxic effects.

We also examined the reliability of the self reports of the mothers since we grouped the infants according to these data. The sensitivity of self reports of the mothers was 100% and specificity was 89%.

The results of our study showed that tobacco exposure by breastfeeding resulted in higher urinary cotinine levels in infants than observed with passive exposure. Thus, even if a smoking mother does not smoke next to her child, she still causes a significant amount of exposure by breastfeeding. Usually, smoking mothers and sometimes healthcare providers think that avoiding passive smoke exposure is enough to prevent the hazardous effects of tobacco smoke exposure. In a previous report of Yılmaz et al.[14], which was also evaluated in a meta-analysis by Priest et al.[15], it was stated that discussion during short pediatric visits on the effects of smoking on a child's health or on maternal health may result in a significant smoking cessation, smoking location change rate or knowledge change. Thus, it is an important duty of healthcare providers, who know the important effect of tobacco exposure by breastfeeding, to encourage mothers to stop smoking particularly during the breastfeeding period.

In conclusion, tobacco exposure via breast-milk led to 13.4 times greater urinary cotinine levels than passive exposure, which suggests that tobacco exposure by breastfeeding can be more harmful than other routes of exposure. Thus, mothers should be encouraged to stop smoking especially during the breastfeeding period, even if they avoid exposing their infant to passive tobacco smoke. The results of our cross-sectional study are preliminary and should be supported by further prospective studies.

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