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An Epidemiologic Study Comparing Fetal Exposure to Tobacco Smoke in Three Southeast Asian Countries

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Abstract

The high prevalence of smoking in Southeast Asia (SEA) means pregnant women face exposure to tobacco smoke that may affect the health of their fetus. This study determined fetal exposure to tobacco smoke by meconium analysis for cotinine in 3 locations in SEA: Bulacan Province, Philippines (N=316), Bangkok, Thailand (N=106) and Singapore City (N=61). Maternal exposure to tobacco smoke was 71.1% (1.3% active; 69.8% passive) in Bulacan, 57.5% (0.9% active; 56.6% passive) in Bangkok and 54.1% (11.5% active; 42.6% passive) in Singapore. Fetal exposure to tobacco smoke (by meconium analysis) was 1.3% (Bulacan), 4.7% (Bangkok) and 13.1% (Singapore); however, a large proportion of infants who tested positive for cotinine (65%) were born to mothers who gave no history of either active or passive exposure to environmental tobacco smoke. Fetal exposure to tobacco smoke is a major health problem.

Keywords

smoking; tobacco; pregnancy; fetus; meconium analysis; cotinine; epidemiology

There is a high prevalence of smoking in Southeast Asia, particularly among men.¹ Although the rate of smoking is lower among females,^{1–3} women, including pregnant women, are likely to be highly exposed tobacco smoke. The hazards of fetal exposure to tobacco smoke are well known,^{4–15} but objective measures to determine the rate of such exposure in the fetus are lacking. The aim of this study was to compare fetal exposure to tobacco smoke using the analysis of meconium for cotinine, a major metabolite of nicotine, as a biomarker of fetal exposure to tobacco smoke. The study was conducted in three areas of Southeast Asia: Bulacan Province, Philippines; Bangkok, Thailand; and Singapore City. To our knowledge, this is the first study to use cotinine analysis of meconium as a biomarker of fetal tobacco exposure.

Methods

The study sites included the Bulacan Provincial Hospital in Malolos, Bulacan, Philippines (N=316), Siriraj Hospital in Bangkok, Thailand (N=106) and the KK Women's and Children's Hospital in Singapore City, Singapore (N=58). Bulacan is a province in the Philippines

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immediately north of Manila and has been the site of an ongoing study of fetal exposure to environmental toxins, including tobacco smoke.

The subjects were pregnant women who delivered at these hospitals and their newborn infants. Informed consent was obtained from the mothers for their participation in the study. Clinical information on the mothers and their infants was obtained from their medical records, as well as by maternal interview. History of maternal exposure to tobacco, whether by active or passive smoking (at home or at work) was obtained by interview. Fetal exposure to nicotine in tobacco smoke was determined by analysis of the infant's meconium for cotinine, which is a principal metabolite of nicotine. Meconium was collected directly from the infants' soiled diapers, pooled and stored at -18°C until the time of analysis. Meconium was collected from first bowel movement through the infants' discharge from hospital. The study was approved by the Human Investigation Board of each participating institute.

Meconium was analyzed for cotinine, the principal metabolite of nicotine, by enzyme linked immunosorbent assay (ELISA) using the Cotinine Urine Micro-Plate EIA (OraSure Technologies, Inc., Bethlehem, PA), adapted for meconium analysis, as follows: 0.5 g of each meconium sample was weighed and placed into Sarstedt tubes (with stirrers). Five mL of (0.1 M) phosphate buffer (pH=6.0) was added to the sample. For the high and low meconium controls, 0.5 g each of cotinine-negative meconium was used to which 4.95 or 4.90 mL of phosphate buffer was added. The samples were vortexed, with stirrer in place, to disperse the meconium in solution. The meconium controls were spiked with 50 or 100 μL of cotinine calibrator (5000 ng/mL) to make a final cotinine concentration of 50 ng/mL or 100 ng/mL. The tubes were vortexed and centrifuged at 4600 rpm for 30 minutes. The supernatant was transferred to a transport tube by a glass Pasteur pipette. Each tube was vortexed and 1.5 mL of the supernate was transferred to a Micropore ultrafilter and centrifuged at 4600 rpm for 30 minutes. The ultrafiltrate was collected and analyzed for cotinine by ELISA. In spiked meconium samples, the coefficient of variation samples ranged from 7.58–11.1% and recovery ranged between 91.22–115.9%. Our criteria for the acceptability of batch test results was a coefficient of variation of <10% and recovery rate of 80–120% of the positive control concentrations.

Since nicotine is also derived from sources other than tobacco, such as tea, tomato, potato, etc.,¹⁶ a correction factor was used to adjust for the effect of these potential confounders on the cotinine concentration in meconium. Based on a previous study, a mean cotinine concentration of 10.9 ng/mL was found in meconium of infants whose mothers were not exposed to tobacco smoke, which could be attributed to maternal exposure to non-tobacco sources of nicotine.¹⁷ Thus, for this study, we used a baseline of 10.9 ng/mL as the cut-off concentration of cotinine in meconium and only values above this concentration level were considered as a positive test for cotinine in meconium that could be attributed to fetal exposure to tobacco smoke.

Statistical Analysis

Descriptive analysis of data was expressed as mean (standard deviation), range and percentage, where appropriate. For cotinine concentration in meconium, "corrected cotinine" was used (see above) and was analyzed as a categorical (yes/no) or continuous variable. For continuous variables, with normal distribution, comparison of two or three samples means was done by the Student "t" test or one-way analysis of variance with post hoc analysis (Duncan), respectively. For continuous variables with skewed distribution, the Mann-Whitney and Kruskal-Wallis tests were utilized. Bivariate correlation was analyzed by Spearman's rho. For non continuous variables, comparison between groups was done by chi-square analysis. Multiple regression analysis was used to determine the association between potential confounders and cotinine concentration in meconium. A p-value of <0.05 was used as the level of statistical significance

Results

The demographic characteristics of the Bulacan (N=316), Bangkok (N=106) and Singapore (N=61) cohorts are shown in Table 1. Maternal age, parity, and incidence of hypertension, diabetes and alcohol use were significantly higher in the Singapore group; rate of single mothers was significantly higher in the Bulacan cohort and gravidity, rate of Cesarean section, prolonged rupture of the membranes and methamphetamine use were significantly higher among the Bangkok mothers. The characteristics of the infants at birth are shown in Table 2. The birth weight, head circumference and incidence of jaundice were significantly higher among the Singapore infants, whereas the incidence of transient tachypnea of the newborn and sepsis were higher among the Bangkok infants and Bulacan infants, respectively.

Table 3 shows the tobacco smoke exposure data. The overall self-reported incidence of active smoking was low, although significantly higher among the Singapore mothers compared to the other groups (11.5% vs 1.3% vs 0.9%, $p<0.001$). Exposure to second hand smoke was high: 42.6% in Singapore, 56.6% in Bangkok and 69.8% in Bulacan. The overall positive test for cotinine in meconium was 3.5% (17/482), with the highest incidence seen in Singapore compared to the other groups (13.1% vs. 4.7% vs. 1.3%, $p<0.001$). The overall mean concentration of cotinine in positive meconium samples was 27.31 ± 16.4 ng/mL (range: 11.44–61.85 ng/mL) and the highest concentration was seen in the Singapore cohorts, although the difference did not achieve statistical significance ($p=0.567$, Kruskal Wallis test). The mean cotinine concentration in meconium was significantly higher in infants whose mothers were active smokers compared to passive or nonsmokers (12.8 ± 20.71 versus 1.67 ± 7.48 versus 0.1162 ± 1.51 ng/mL, respectively $p<0.001$, Kruskal-Wallis test). Similarly, in the cotinine-positive group, there was a higher mean concentration of cotinine in meconium in infants whose mothers were active smokers (38.37 ± 16.24 ng/mL, N=4) compared to the passive (17.77 ± 8.22 ng/mL, N=2) or nonsmoker (25.02 ± 16.45 ng/mL, N=11), although the difference did not achieve statistical significance ($p=0.153$, Kruskal-Wallis test). Of note, 64.7% (11/17) of infants with cotinine-positive meconium were born to mothers with no known active nor passive exposure to tobacco smoke at home or in the workplace; the mean cotinine concentration in this group was 25.01 ± 16.44 ng/mL (range: 11.44–54.83 ng/mL). Factors that were significantly ($p<0.001$) related to positive meconium test for cotinine included country of origin, maternal gravidity, parity and hypertension. There was a significant correlation between the number of cigarettes smoked per day and cotinine concentration in the entire cohort ($r=0.260$, Spearman's rho, $p<0.001$), although in individual sites, the correlation was only significant in the Bulacan cohort ($r=0.716$, Spearman's rho, $p<0.01$). With cotinine values continuously scaled, mean increases or decreases in cotinine values were looked at as a result of which variables loaded significantly in a multiple regression model. Collectively, number of cigarettes per day ($R^2=0.095$), parity ($R^2=0.048$), and exposure to passive smoke ($R^2=0.043$) loaded significantly into the final multiple linear regression model ($R^2=0.186$). On average, increasing the number of cigarettes per day by one increased the corrected cotinine value by 2.14 (95% CI: 1.22–3.05; $p\leq 0.001$) as the single best predictor of an increase in cotinine. In the final model, a one unit increase in parity increased cotinine by 1.64 units ($p\leq 0.001$), and a one unit increase (being exposed to passive smoking as opposed to not) increased the cotinine value by 3.65 units ($p\leq 0.001$).

There was no significant difference in mean gestational age, birth weight, length and head circumference between cotinine-positive and negative infants (Table 4).

Discussion

The prevalence of smoking in many Southeast Asian countries is high and according to available estimates, the highest numbers of smokers in Southeast Asia are in Vietnam,

Cambodia, South Korea, Indonesia, China and the Philippines.¹ The Western Pacific accounts for one-third of the cigarettes smoked in the world. Countries in Asia have widespread smoking, especially among men, although the rate of smoking among women has recently increased.^{2,3}

Smoking poses a significant health problem, and in pregnant women, exposure to tobacco smoke is deleterious to the health of both the mother and her fetus. According to a report by the US Surgeon General, smoking is probably the most important modifiable cause of poor pregnancy outcome among women in the United States.⁴ Nicotine, carbon monoxide and other toxic constituents of tobacco smoke readily cross the placenta and have a direct, adverse effect on oxygen supply to the fetus, structure and function of the umbilical cord and placenta, fetal heart rate and fetal breathing.^{4–6} Reports of complications in pregnancy that have been associated with exposure to tobacco smoke include higher incidence of spontaneous abortions, ectopic pregnancy, prematurity, low birth weight, and increased fetal and infant death.^{4–13} Long-term effects in the infant and child have included impairment of behavior, intelligence and physical characteristics, and attention deficit/hyperactivity disorder.^{14,15}

In this study, the self-reported rates of active smoking among women during pregnancy in the Bulacan and Bangkok cohorts were low (0.9–1.3%) as compared to 11.5% in Singapore ($p < 0.001$). The latter is consistent with reported increasing rate of smoking among women in Singapore.³ On the other hand, the self-reported rate of exposure to second hand smoke in the household or workplace was high in all groups, ranging from 42.6% in Singapore to 69.8% in Bulacan. The sources of exposure at home were principally from the husband and/or other household members, such as grandparents.

The analysis of meconium for cotinine, a metabolite of nicotine, was used in this study as a biomarker of fetal exposure to tobacco smoke. Meconium is a sensitive matrix to analyze for fetal exposure to many xenobiotics, since compounds that cross the placenta from the mother to the fetus are metabolized and deposited in meconium either through the bile secretion or fetal swallowing of fetal urine via the amniotic fluid.¹⁹ Since meconium accumulates throughout gestation, starting at the 12th week of gestation, it is a significant repository of compounds to which the fetus is exposed during a large part of gestation. Meconium is therefore an ideal matrix to analyze for fetal exposure to various xenobiotics.^{17–26} Meconium analysis of nicotine and its metabolites, principally cotinine, has been established as a valuable and sensitive measure of fetal exposure to tobacco smoke.^{17,27–31}

Since nicotine can also be derived from plant sources other than tobacco, such as tomato, potato and tea,¹⁶ a correction factor of 10.9 ng/mL was used in this study to adjust for this confounder. This value was obtained from the only available study in the literature, which provided a mean concentration of cotinine in meconium in infants whose mothers were not known to be actively or passively exposed to tobacco smoke.¹⁷ Thus, any concentration of cotinine in meconium that was above 10.9 ng/mL was considered as a positive test in this paper. Although the use of a correction factor may underestimate the true incidence of fetal exposure to tobacco smoke, it appears that the difference is only slight, since omission of the correction factor would have only increased the incidence of cotinine-positive meconium from 1.3% to 3.8% for the Bulacan cohort, from 4.7% to 10.4% for the Bangkok cohort and from 13.1% to 14.0% in the Singapore cohort.

The overall rates of cotinine-positive meconium were 1.3%, 4.7% and 13.10% in the Bulacan, Bangkok and Singapore cohorts, respectively (Table 3). There was a wide range of cotinine concentrations in meconium. Concentrations were directly related to the manner of exposure to tobacco smoke (active or passive) and, in active smoking, to the number of cigarettes smoked per day. Although there was a high rate of exposure to tobacco smoke, the rate of positive

meconium test for cotinine was comparatively lower (Table 3). We attribute this likely due to marked variations in the degree of exposure to tobacco smoke in the mother, particularly in passive smoking. This finding underscores the need for fetal biomarkers as more objective and reliable measures of fetal exposure to tobacco compared to questionnaire or history-taking alone. It is noteworthy that a large percentage (64.7%) of the cotinine-positive infants in the study were born to mothers with *no reported exposure* to tobacco smoke at home or workplace. It is likely that passive exposure to tobacco smoke in public places may be an important avenue of exposure to tobacco smoke. This is particularly worrisome since second hand smoking has been shown to be as hazardous to health as active smoking.³² In fact, in a previous study, we have demonstrated that the concentrations of cotinine in meconium were not significantly different between groups of infants whose mothers smoked 1 pack of cigarette per day during pregnancy compared to passive smokers alone.¹⁷

We have looked at other factors that correlated with positive meconium tests for cotinine and found that the study site, maternal gravidity and parity and number of cigarette smoked were significantly related to or associated with a positive cotinine test. In a multiple regression model, the number of cigarettes smoked per day, parity, and exposure to passive smoke loaded significantly into the final multiple linear regression model for cotinine concentration.

Although smoking has been shown in many studies to have an adverse effect on infant birth weight, length and head circumference,^{33,4} we did not observe this relationship in the study (Table 4), most likely due to the small sample size of the positive group. Thus, a larger cohort of infants with cotinine-positive meconium will be needed to establish the true relationship of these variables to the outcome.

There are some limitations to this study. We did not analyze any maternal tissue that could have served a biomarker of maternal exposure to tobacco smoke, since our paper was focused on a fetal biomarker of exposure and meconium has been shown in many studies to be one of the best matrices to analyze for this purpose. However, for future studies, the relationship between maternal and fetal biomarkers of tobacco exposure can be compared, particularly in relation to maternal exposure to tobacco smoke, whether active or passive. Likewise, we were not able to obtain information on other food sources of nicotine that could be important confounders for cotinine in meconium. Although we introduced a correction factor for cotinine in this study that enabled us to approximate cotinine in meconium from tobacco, future studies will be designed to consider this variable. Finally, the failure in this study to show known adverse effects of smoking on infant anthropometry at birth may be due to the small number of cotinine-positive infants in the study. Thus, a larger and more balanced sample size is warranted for future studies.

Conclusion

Our study in three selected regions in Southeast Asia showed, by analysis of meconium for cotinine, a 3.5% rate of fetal exposure to tobacco smoke, principally from passive smoking. Noteworthy also was the large percentage of infants with cotinine-positive meconium who were born to mothers with no evident exposure to tobacco smoke in their homes or work place. Exposure to environmental tobacco smoke in public places, other than home or workplace, is a plausible cause. Thus, measures should be vigorously undertaken not only in discouraging pregnant women as well as the general public from active smoking but also in preventing exposure to environmental second hand smoke, especially in public areas. This study is unique because it utilized a fetal biomarker, through analysis of cotinine in meconium, to determine fetal exposure to tobacco smoke.

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Table 1
Maternal Demographics of the Study Population

	Bulacan	Bangkok	Singapore	p
N	315	106	61	
Maternal age in yrs—mean (S.D.)	25.5 (5.7)	26.3 (6.2)	30.2(5.4) *	0.05
Single (%)	26.3 **	7.3	4.3	0.001
Gravida	2.3 (1.5)	1.8 (0.9) **	2.3 (1.4)	0.05
Parity	1.2 (1.4)	1.6 (0.7)	2.0 (1.2) *	0.001
Term	1.1 (1.4)	1.3 (0.7)	1.9 (1.2) *	0.05
Preterm	0.03 (0.18)	0.22 (0.46) *	0.13 (0.43)	0.05
Abortion	0.13 (0.41)	0.27 (0.53) *	0.26 (0.75) *	0.05
Meconium-stained amniotic fluid (%)	13.7	17.9	11.5	ns
No prenatal care (%)	1.6	5.7	3.3	ns
Oligohydramnios (%)	1.8	3.8	0.0	ns
Breech presentation (%)	4.2	4.7	11.5	ns
Cesarean section (%)	17.6	36.8 **	27.9	0.001
Prolonged rupture of membranes (%)	1.9	14.2 **	3.3	0.001
Placenta praevia (%)	0.0	0.9	1.6	ns
Hypertension (%)	3.2	6.6	6.6 **	0.001
Diabetes mellitus (%)	1.0	6.6	10.0 **	0.001
Cocaine use (%)	0.0	0.0	0.0	ns
Opiate use (%)	0.3	0.0	0.0	ns
Marijuana use (%)	1.0	0.0	0.0	ns
Methamphetamine use (%)	1.0	3.8 **	1.6	0.023
Alcohol use (%)	0.6	0.9	3.6 **	0.001

* by one way analysis of variance

** by Chi-square analysis

ns—not significant

Table 2
Characteristics of infants in the study population

	Bulacan	Bangkok	Singapore	p
N	315	106	61	
Gestation (wks)—mean (SD)	38.4 (2.1)	38.1(2.0)	38.5.(1.6)	ns
Male (%)	56.2	56.7	55.7	ns
Weight (g)	2888.6 (413.6)	2951.7 (582.7)	3092.0 (499.4) *	0.050
Length (cm)	48.8 (2.7)	48.6 (2.6)	48.6 (1.9)	ns
Head circ (cm)	33.0 (1.6)	32.8 (1.6)	33.8 (1.5) *	0.050
Respiratory distress syndrome (%)	0.6	0.9	1.6	ns
Transient tachypnea of newborn (%)	1.6	4.7 **	3.3	ns
Meconium aspiration syndrome (%)	0.6	1.9	0.0	ns
Asphyxia (%)	1.9	0.0	0.0	ns
Jaundice (%)	5.7	0.9	13.1 **	0.004
Sepsis (%)	10.5 **	0.0	0.0	0.001
Necrotizing enterocolitis (%)	0.3	0.0	0.0	ns

* by one way analysis of variance

** by Chi-square analysis

ns—not significant

Table 3
Comparison of exposure to tobacco smoke in Bulacan (Philippines), Bangkok (Thailand) and Singapore

	Bulacan (N=315)	Bangkok (N=106)	Singapore (N=61)	Total	p
Exposure to tobacco smoke	71.1%	57.5%	54.1%		
Active smoking	1.3%	0.9%	11.5% *	2.5%	0.001
Second hand smoking	69.8% *	56.6%	42.6%	63.5%	0.001
Meconium positive for cotinine (%)	1.3%	4.7%	13.1% *	3.5%	0.001
Mean (SD) cotinine concentration in positive meconium samples (ng/mL)	29.33 (23.5)	23.63 (17.48)	28.60 (13.71)	27.31 (16.38)	0.567 **

*
by Chi square analysis

**
Kruskal-Wallis

Table 4
Comparison, by Region, of Infants' Mean Birth Weight, Length and Head Circumference in Relation to Prenatal Exposure to Tobacco Smoke (Positive/Negative for Cotinine in Meconium) *

	Cotinine in Meconium	N	Mean	SD	* p
Gestational age (wks)	Negative	461	38.3	2.0	0.787
	Positive	17	38.5	1.9	
Birth weight (g)	Negative	461	2926.8	463.7	0.725
	Positive	17	2967.7	648.5	
Length (cm)	Negative	461	48.7	2.6	0.281
	Positive	17	48.0	2.6	
Head circumference (cm)	Negative	461	33.1	1.6	0.154
	Positive	17	33.6	2.2	

* Student "t" test