Original Investigation

Measuring prenatal secondhand smoke exposure in mother–baby couplets

Kristin B. Ashford, Ellen Hahn, Lynne Hall, Mary K. Rayens, Melody Noland, & Rebecca Collins

Abstract

Introduction: Pregnant women often underreport their smoking status and extent of secondhand smoke (SHS) exposure. Biomarker confirmation is the recommended method to assess smoking behaviors and SHS exposure in both mothers and infants.

Objectives: The primary aims are to (a) examine the relationship between smoking behaviors and SHS exposure in mother–baby couplets using maternal and infant hair nicotine and maternal urine cotinine analyses and (b) determine whether there is an association between maternal and infant hair nicotine samples obtained shortly after birth.

Discussion: A cross-sectional study with a multiethnic sample of 210 mother–baby couplets assessing SHS exposure.

Results: The level of maternal hair nicotine (MHN) was significantly different among three groups: nonsmoking, nonsmoking/passive exposed, and smoking ($p < .0001$), with nonsmoking and nonexposed women having the lowest level. Urine cotinine was strongly associated with self-reported smoking status ($p = .88$; $p < .0001$). Maternal and infant hair nicotine were correlated, although MHN correlated more strongly with smoking status ($p = .46$, $p < .0001$) than infant hair nicotine ($p = .39$, $p < .0001$).

Conclusions: MHN was a more precise biomarker of prenatal SHS exposure than infant hair nicotine; mothers' urine cotinine was strongly correlated with self-reported smoking status.

Introduction

There is no debate that tobacco use is the single most modifiable cause of poor pregnancy outcomes; yet health effects of maternal

exposure to secondhand smoke (SHS) during pregnancy are less clear. In 2006, the U.S. Surgeon General reported that there is no safe level of SHS exposure; in fact, SHS exposure causes premature death in infants and adults. SHS exposure increases the risk of respiratory and ear infections, frequency and severity of asthma events in children, and is responsible for approximately 43,000 cases of heart disease and 3,000 cases of lung cancer deaths among nonsmokers each year (U.S. Department of Health and Human Services, 2006). Passive smoking has been reported to impact negatively breast-feeding duration (Jedrychowski et al., 2008) as well as incidence of pediatric dental caries (Aligne, Moss, Auinger, & Weitzman, 2003).

Biomarker validation (Webb, Boyd, Messina, & Windsor, 2003) or a combined assessment of biomarker and self-report measures (Dukic, Niessner, Pickett, Benowitz, & Wakschlag, 2009) are recommended methods to confirm prenatal smoking and SHS exposure status. For social acceptability reasons, many pregnant women underreport or misrepresent their smoking status (Bottorff, Johnson, Irwin, & Ratner, 2000; Dukic et al.) and extent of SHS exposure (DeLorenze, Kharrazi, Kaufman, Eskenazi, & Bernert, 2002). Biomarkers of maternal SHS exposure during pregnancy can be detected in both the mother and the fetus (Eliopoulos et al., 1994; Jacqz-Aigrain et al., 2002; Klein & Koren, 1999; Nafstad et al., 1998).

There are numerous maternal and fetal biomarkers from direct maternal consumption of nicotine and from inhalation of SHS. Frequently collected biomarkers for quantifying maternal smoking and SHS exposure during pregnancy include, but are not limited to salivary cotinine (McBride et al., 1999; Rebagliato, Bolumar, & Florey Cdu, 1995; Van't Hof, Wall, Dowler, & Stark, 2000), serum cotinine (DeLorenze et al., 2002; Kaufman, Kharrazi, Delorenze, Eskenazi, & Bernert, 2002; Kharrazi, DeLorenze, Kaufman, Eskenazi, & Bernert, 2004; Peacock et al., 1998), expired carbon monoxide (Hajek et al., 2001; Johnson, Ratner, Bottorff, Hall, & Dahinten, 2000), urine cotinine

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(Pichini et al., 2000; Webb et al., 2003), hair cotinine (Klein & Koren, 1999), and hair nicotine (Jaakkola, Jaakkola, & Zahlsen, 2001; Pichini et al., 2003). Fetal exposure to nicotine occurs after absorbed nicotine and its metabolite, cotinine, diffuse through the placenta (Sastri, Chance, Hemontolor, & Goddijn-Wessel, 1998). Nicotine/cotinine is then deposited in many fetal/placental tissues and fluids. Common sources for fetal and infant markers of exposure to direct maternal consumption and prenatal SHS exposure include amniotic fluid, cord blood, meconium, urine, and hair (Klein, Chitayat, & Koren, 1993). Infant hair analysis is a reliable biomarker for SHS exposure and maternal smoking in infants (Eliopoulos et al., 1994; Jacqz-Aigrain et al., 2002; Klein & Koren).

Nicotine in maternal hair is the biomarker most strongly associated with parental reports of SHS exposure (Sorensen, Bisgaard, Stage, & Loft, 2007). In the past decade, several studies chose hair as a biomarker to examine passive smoke exposure during pregnancy but differed in use of cotinine or nicotine (Jaakkola et al., 2001; Klein, Blanchette, & Koren, 2004; Klein & Koren, 1999; Nafstad et al., 1998; Pichini et al., 2003; Sorensen et al., 2007). In a recent study by Moon-Woo et al. (2008), maternal and paternal hair nicotine/cotinine concentrations were compared with neonatal hair. Nicotine proved a more useful marker of neonatal exposure to paternal smoking compared with cotinine. The advantage of nicotine over cotinine for hair analysis can be explained by the increased concentrations of nicotine over cotinine in neonatal hair (Gerstenberg, Schepers, Voncken, & Volkel, 1995; Moon-Woo et al.). Other studies report nicotine to be a more valid and useful biomarker compared with hair cotinine (Al-Delaimy, Crane, & Woodward, 2002; Dimich-Ward, Gee, Brauer, & Leung, 1997; Pichini, Altieri, Pellegrini, Pacifici, & Zuccaro, 1997).

Collection of maternal and infant hair for cotinine/nicotine analysis in mother–baby couplets is not new. Of the couplet research using hair nicotine, half of the studies found moderate to strong correlations between mother–infant nicotine levels (Eliopoulos et al., 1994; Klein & Koren, 1999). Others found no correlation between maternal and infant hair nicotine (Jacqz-Aigrain et al., 2002; Nafstad et al., 1998). Although cutting of hair, maternal or infant, can have cultural implications, it did not pose an obstacle in this study. The purpose of this article is to analyze the validity and reliability of biomarker and self-report measures of prenatal SHS exposure in mother–baby couplets. The primary aims are to: (a) examine the relationship between self-reported smoking behaviors and SHS exposure in mother–baby couplets using maternal and infant hair nicotine and maternal urine cotinine analyses and (b) determine if there is an association between maternal and infant hair nicotine samples obtained shortly after birth.

Methods

A cross-sectional study design was used to investigate the relationships of self-reported smoking and prenatal SHS exposure, with maternal urine cotinine and mother–baby hair nicotine levels in the immediate postpartum period. There were two hypotheses: (a) self-reported maternal smoking and SHS exposure will be positively correlated with maternal and infant hair nicotine and maternal urine cotinine and (b) maternal and infant hair nicotine will be positively associated shortly after birth. Quota

sampling was used to ensure a representative distribution of mothers who were smokers, nonsmokers/passively exposed, and nonsmokers/nonexposed during pregnancy. Twenty-four percent of pregnant women in Kentucky smoke cigarettes (Centers for Disease Control and Prevention, (CDC), 2004). Since approximately 60% of the accessible population was Medicaid recipients who have a 50% greater smoking prevalence than the overall U.S. population (CDC), about 60 mothers were expected to be smokers (approximately 30% of the total sample). The remaining 140 pregnant women were recruited based on nonsmoking status and self-reported exposure. A statewide U.K. Health Care survey (2006) reported that 34% of Kentuckians allow smoking in their homes. Of the nonsmokers, it was expected that at least 48 would be exposed to SHS (35% of nonsmoking subsample) and 92 would not be exposed.

Data collected on self-reported SHS exposure and other information included: number of days or hours exposed to smoking in the home, work, or vehicle in the past 7 days; number of persons smoking in the home; and information on cosmetic perms, straighteners, bleaching, and hair dye. Total SHS exposure was defined as the total number of hours per week of SHS in the home, vehicle, and at work. A woman was classified as a self-reported smoker if she responded “yes” to the question, “Have you smoked a cigarette, even a puff, in the past 7 days.” Mothers who smoked were asked to classify their daily smoking consumption over the past 30 days as: <1 cigarette, 1–5, 6–10, 11–15, 16–20, 21–25, 31–35, 36–40, and >40 cigarettes.

To assess SHS exposure, average daily number of cigarettes smoked for each family member and visitor (within the past week) was calculated based on the following five categories: 1–5, 6–10, 11–15, 16–20, and >20 (Al-Delaimy et al., 2002). Number of exposure sources was calculated by adding home, car/vehicle, and work exposures (0–3). For example, respondents reporting no days or hours of home, work, or vehicle exposure were coded “0”; if respondents were exposed at home, work, and in their vehicle, they were coded “3” (Okoli, Hall, Rayens, & Hahn, 2007). If the participant did not report any exposures to any of the sources, they were classified as nonsmoking, nonexposed (NS/NE). Classification of SHS exposure (in confirmed nonsmokers) was based on self-report. If a participant answered “yes” or quantified exposure (hours or days) to any of the smoking exposure questions, they were classified as “nonsmoking, passive-exposed” (NS/PE).

Collection of maternal hair involved cutting a proximal segment of hair from the posterior vertex of the scalp. The hair segment (approximately 20–25 strands) was cut as close as possible to the scalp and placed in a paper envelope. For analysis, duplicate groups of 1–2 cm lengths were cut. Because human hair grows approximately 1 cm per month, this length of hair reflects exposure for the previous 1–2 months (Zahlsen & Nilsen, 1994). Collection of infant/child hair involved cutting approximately 10–50 mg of hair (a pencil-width segment) from the scalp behind the ear or at the occipital region (Al-Delaimy et al., 2002; Klein & Koren, 1999; Pichini et al., 2003).

Matched hair samples were placed in a paper envelope and stored until being mailed to Wellington Hospital, Wellington, New Zealand, for analysis. Hair samples can be stored without deterioration for at least 5 years (Zahlsen & Nilsen, 1994). Nicotine was extracted from the hair and measured using the method of Mahoney and Al-Delaimy (2001) with the following

Table 1. Demographic characteristics and smoking status

	Smoker		Passive/nonexposed		Nonsmoker/nonexposed	
Ethnic group, <i>N</i> = 210	<i>n</i> = 91 (%)		<i>n</i> = 53 (%)		<i>n</i> = 66 (%)	
White	45 (21.4)		41 (19.5)		33 (15.7)	
Black	8 (3.8)		10 (4.8)		14 (6.7)	
Hispanic	35 (16.7)		0		18 (8.6)	
Asian	2 (1)		0		0	
Other	1 (0.5)		2 (1)		1 (0.5)	
Marital status, <i>n</i> = 209						
Single	25 (12)		34 (16.3)		35 (16.7)	
Married	64 (30.6)		15 (7.2)		26 (12.4)	
Separated	2 (1)		3 (1.4)		5 (2.4)	
Highest grade completed, <i>n</i> = 209						
Less than high school	22 (11.1)		13 (6.2)		23 (11.0)	
High school/GED	17 (8.1)		25 (12)		20 (9.6)	
Some college and above	14 (6.7)		27 (12.9)		48 (23)	
Total household income, <i>n</i> = 172						
Less than \$20K	34 (19.8)		32 (18.6)		24 (14)	
\$20K–\$39.9K	6 (3.5)		10 (5.8)		24 (14)	
\$40K and above	2 (1.2)		10 (5.8)		30 (17.4)	
Other characteristics	<i>SD</i>		<i>SD</i>		<i>SD</i>	
Age (years)	24.8	5.11	24.3	5.15	27.2	5.37
Adults smoking inside > 1 month	2.4	1.95	1.2	1.35	0.1	0.31
Total hours passive smoke (hours calculated per week)	79.8	53.22	45	40.98	0	0

modifications. Each hair sample was cleaned thoroughly to remove any nicotine on the outside of the hair prior to analysis. Digestion was accomplished using a 2-hr incubation at 70–75 °C and, after ether extraction, nicotine was quantified by high-performance liquid chromatography (HPLC) with electrochemical detection using a Phenomenex (Torrance, CA), 5 μ , C18, 150 $\times$ 4.6-mm Gemini column and mobile phase consisting of 0.075 mol/L potassium phosphate buffer, pH 4.8, with 4% acetonitrile and 7.5% methanol added. Within and between batch, imprecision was less than 10%. The limit of detection was 0.05 ng/mg hair, and the more relevant parameter for our analysis, the limit of quantitation was about 0.3 ng/mg hair.

After collection of maternal and infant hair, a urine sample was obtained from the mother for validation of smoking status using NicAlert, a commercial urine assay, with cutoff limits of urine cotinine levels (NicAlert, 2007). NicAlert measurement correlates well with more complex laboratory tests using HPLC used in the CDC laboratory (Bernert, Harmon, Sosnoff, & McGuffey, 2005). Nonsmokers were defined by urine cotinine <99 ng/ml (level 00–02). Current smokers were defined by urine cotinine >100 ng/ml (level 03–06). Bernert et al. reported classification sensitivity and specificity were 88% and 92%, respectively, for cotinine measured by NicAlert. NicAlert cutoffs for smoking validation are consistent with previous reported urine cotinine ranges (Higgins et al., 2007). While every effort was made to minimize the number of missing values for hair nicotine or urine cotinine, some incomplete data were unavoidable due to inability in some cases to collect or analyze hair or discharge prior to urine sample collection. Participants' missing data for these measures were not included in the analysis. Details on the extent of missing data are described in the Results section.

The study was approved by the University of Kentucky Medical Institutional Review Board. After obtaining consent, research assistants administered the 20-min questionnaire and collected urine and hair samples. The questionnaire was also written in Spanish (6th-grade level) and administered by trained medical translators. Content verification of all written study materials was conducted by two expert translators. Participants were offered a choice of two incentives to participate: a one-time payment of \$25 or the equivalent of \$25 in diapers and baby wipes.

Data analysis and power calculations

Raw hair nicotine data were log transformed to normalize the distributions. The geometric means (GMs, i.e., the antilogs of the means of the log-transformed data) were used to convert means of the log-transformed data back to the original scale. Univariate analyses were used to summarize demographic and socioeconomic characteristics of the participants (see Table 1). To determine the strength of the correlations between self-reported SHS exposure and biomarkers and the relationship between nicotine levels in mothers and their infants, Spearman's rank order correlation was used. When there was a discrepancy between self-reported smoking status and urine cotinine, biomarker data were used to indicate maternal smoking status. Nonparametric tests (Mann–Whitney for two-group comparisons and Kruskal–Wallis for more than two groups) were used to assess the difference in mother–baby hair nicotine levels according to smoking and SHS exposure, ethnicity, and hair condition. Multiple linear regression was used to predict the direction and magnitude of the relationship between levels of maternal and infant hair nicotine and other independent variables while adjusting for potential confounders, such as maternal

Table 2. Comparisons of self-reported smoking/exposure status and biomarkers of exposure

	Nonsmoking/ nonexposed	Nonsmoking/ exposed	Smoking
Biomarkers (n)	91	66	53
Maternal urine cotinine (ng/ml)	<99	<99	500–2,000
Mother hair nicotine (ng/ml)			
n	90	66	52
Median	0.3	0.74	9.81
GM	0.33	1.02	9.6
95% CI	0.27–0.40	0.72–1.44	7.27–12.69
Infant hair nicotine (ng/ml)			
N	65	61	48
Median	0.38	0.34	0.86
GM	0.32	0.33	0.76
95% CI	0.27–0.38	0.28–0.39	0.65–0.96

Note. GM = geometric means.

age, education, and ethnicity. The regression R^2 was assessed to determine the variability in mother and baby hair nicotine predicted by the exposure variables. Data were analyzed using SPSS version 16.0, an alpha level of .05 was used throughout.

Results

Of the 210 mothers, 53 were smokers (25%) and 157 were nonsmokers (75%). Of the nonsmokers, 66 reported exposure to SHS (42% of nonsmoking subsample) and 91 were nonsmokers, nonexposed to SHS during pregnancy. A multiethnic sample was recruited: Caucasian (57%), Hispanic (25%), Black (15%), Asian (1%), and multiethnicity (1%). All mothers were between the ages of 18 and 40 years, with a mean age of 26 ($SD = 5.4$) years; 42% were educated beyond high school, and 55% had a family income of \$30,000 or less per year. On average, infants were born at 38 weeks gestation, weighed 3,159 g, were 49.9 cm in length, and had 1-min, 5-min Apgar scores of 8. There were more male infants (57%) than female infants (43%), and 43 (20%) infants were born premature. Study population demographic characteristics and smoking status are summarized in Table 1.

Hair samples from 209 postpartum women and 198 infants were obtained. Hair was not collected on 10 infants (5%) due to lack of hair or critical medical status. One mother–baby couplet was discharged prior to collection of hair samples. Hair nicotine was determined in nearly all maternal hair samples and in the majority of infant samples (88%). In prior research, as little as 30% of infant hair samples were quantifiable (Pichini et al., 2003). In total, 208 maternal and 174 infant hair samples were analyzed.

A summary of the relationships of smoking status with maternal urine cotinine and mother–baby couplet hair is shown in Table 2. Women who do not smoke had urine cotinine levels <99 ng/ml compared with smoking mothers whose urine cotinine levels ranged from 500 to 2,000 ng/ml. A significant asso-

Figure 1. Geometric means and 95% CIs, by active/passive smoking status of mother.

ciation was found among the three smoking/exposure categories and urine cotinine measurement (Kruskal–Wallis, $\chi^2_{df=2} = 160.82$; $p < .0001$); however, the association between self-reported exposure status and urine cotinine was not significant. One fourth of women had positive urine cotinine (25.2%) and a slightly higher percentage identified themselves as smokers (28.5%). No self-reported nonsmoking/nonexposed women had urine cotinine levels >100 ng/ml.

The level of maternal hair nicotine (MHN) was significantly different among the three groups: NS/NE, NS/PE, and smoking (Kruskal–Wallis, $\chi^2_{df=2} = 116.67$; $p < .0001$; see Figure 1). There was a strong correlation between urine cotinine (NicAlert level) and self-reported smoking status ($\rho = .88$; $p < .0001$). The correlation was slightly stronger between MHN and smoking status compared with infant hair nicotine ($\rho = .46$, $p < .0001$ for maternal hair; $\rho = .39$, $p < .0001$ for baby hair). Maternal and baby hair nicotine measurements were positively correlated ($\rho = .46$, $p < .0001$). The association between urine cotinine and MHN was positive and significant ($\rho = .63$, $p < .0001$). Correlations between self-reported smoking variables and each of maternal and baby hair nicotine values are

Table 3. Spearman rank correlations between hair nicotine and self-reported smoking variables

Self-reported smoking variables	Log-transformed hair nicotine	
	Maternal hair	Infant hair
Ordinal smoking status variable	.74*	.39*
Number of cigarettes per day	.68*	.45*
Total SHS exposure*	.68*	.28*
Number of adults smoking in home	.66*	.27*
SHS home exposure (hours/week)	.65*	.02
SHS home and car/vehicle (hours/week)	.63*	.27*
Number of exposure sources	.58*	.19*
SHS car/vehicle exposure (hours/week)	.46*	.07

Note. SHS = secondhand smoke. Total SHS exposure includes home, work, and vehicle (hours/week).

*Significant at the .01 level.

Table 4. Multiple regression estimates for the best predictive model of hair nicotine (mother *N* = 186; infant *N* = 158)

Variable	Mother-active smoker excluded			Mother-active smoker included			Infant-active smoker excluded			Infant-active smoker included		
	<i>B</i>	<i>SE</i>	β	<i>B</i>	<i>SE</i>	β	<i>B</i>	<i>SE</i>	β	<i>B</i>	<i>SE</i>	β
Age	−0.02	0.02	−0.07	0.00	0.02	−0.01	−0.02	0.01	−0.11	−0.01	0.01	−0.07
Highest grade completed	−0.33	0.09	−0.26**	−0.22	0.07	−0.17**	0.00	0.05	−0.01	0.03	0.05	0.05
White	−0.08	0.49	−0.02	−0.22	0.41	−0.06	0.22	0.36	0.14	0.13	0.35	0.08
Black	0.79	0.52	0.17	0.58	0.45	0.13	0.43	0.38	0.22	0.33	0.37	0.16
Hispanic	−1.15	0.54	−0.26*	−0.87	0.46	−0.20	−0.10	0.39	−0.05	−0.06	0.38	−0.03
Passive Smoker	−0.46	0.19	−0.13**	0.67	0.21	0.18**	−0.47	0.12	−0.29**	−0.17	0.16	−0.11
Total hours passive smoke	0.01	0.00	0.30**	2.32	0.28	0.59**	0.00	0.00	0.27**	0.59	0.21	0.35**
Adults smoking inside > 1 month	0.34	0.06	0.31**	0.15	0.06	0.14**	0.02	0.04	0.05	−0.02	0.04	−0.05
<i>R</i> ²		0.59			0.70			0.23			0.27	
<i>F</i> for change in <i>R</i> ²		31.33**			46.17**			5.68**			6.20**	

Note. *Significant at .05.

**Significant at .01.

summarized in Table 3. While both associations are significant at the .0001 level, the correlation between exposure and hair nicotine was stronger for maternal measurements ($\rho = .68$) than for baby measurements ($\rho = .28$). Smoking mothers' median hair nicotine content was more than 30 times higher than that of NS/NE mothers and 14 times higher than NS/PE mothers. Infant hair nicotine samples also displayed noteworthy findings when comparing mothers who smoke and mothers who do not smoke (Kruskal–Wallis, $\chi^2_{df=2} = 44.07$; $p < .0001$). Infants of mothers who smoke had two and half times higher hair nicotine content than infants of mothers who do not smoke. Comparisons between infant hair nicotine in the groups who do not smoke were not as profound. GM for the hair nicotine values and the 95% CI for the GM are also displayed in Table 2. Consistent with the comparisons of the log-transformed values, the GM for the NS/NE mothers and their infants were lower than the GM for the NS/PE and smoking mothers and their infants.

Association between maternal and infant hair nicotine

There was a moderate correlation between mother–baby couplet hair nicotine ($\rho = .46$; $p < .0001$). As shown in Table 3, MHN samples were more strongly correlated with all the self-reported smoking behaviors than were the infant samples. All measured smoking behaviors were significantly correlated with maternal hair samples. The strongest relationship was between MHN and the ordinal smoking status variable, followed by number of cigarettes smoked per day, while vehicle exposure to SHS had the weakest relationship. Only two variables, SHS home exposure and vehicle exposure, were not significantly correlated with infant hair nicotine. Both mother and baby hair nicotine levels were significantly correlated with urine cotinine ($\rho = .62$ and $\rho = .45$, respectively; $p < .0001$).

Predictors of hair nicotine in mother–baby couplets

In the multivariate regression model, age, education, ethnicity, number who smoke in the home, smoking status, and total hours of SHS exposure explained 69% of the variance in MHN (see Table 4). Smoking status was the strongest predictor of MHN level, followed by Hispanic ethnicity, passive smoker (NS/PE), and education. Other variables were not significant.

In infant hair samples, only 27% of the variance in hair nicotine was explained by maternal predictors. Smoking was the only significant predictor of infant hair nicotine. To ascertain the best predictor of hair nicotine levels in relation to SHS exposure variables, the “total SHS exposure” variable, measuring total hours of SHS exposure, was replaced by “total number of SHS sources.” In both models, total hours of SHS exposure were a better predictor of hair nicotine levels than number of sources. Multicollinearity assessments for the total SHS exposure variable did not suggest any confounding with other control variables in the model (variation inflation factor = 1.8).

To be sure that the maternal smoking variable did not overly influence other variable interactions, the regression analysis was replicated with the exception of excluding the maternal smoking variable (see Table 4). Again, the total SHS exposure (hours) variable best predicted hair nicotine level in both mothers and infants. In this model, there was an 11% reduction in *R*² for MHN and a 4% reduction for infant hair nicotine.

Discussion

Fetal exposure to nicotine/cotinine can occur from both direct maternal consumption of tobacco products and indirect maternal exposure to SHS. Most research examines biomarkers from

mothers who smoke while excluding mothers who do not smoke. Although some studies have explored this phenomenon, few have measured maternal and fetal SHS exposure using hair from mother–baby. Analysis of 1 cm of maternal hair typically reflects exposure during the mother’s last month of pregnancy. In this study, levels of nicotine in maternal hair were significantly associated with maternal smoking consumption, consistent with other studies (Jacqz-Aigrain et al., 2002). Pichini et al. (2003) had a novel approach to analyze a 5-cm hair segment, with the first 2.5 cm representing the last 3–4 months of pregnancy and the last segment representing the first 3–4 months of pregnancy. Results indicated that hair nicotine was able to discriminate between exposure and nonexposure to SHS in women who do not smoke.

The differences in level of hair nicotine by smoking status of the mother were more pronounced in maternal hair samples compared with infant hair. We found maternal hair to be a reliable biomarker for measuring direct maternal nicotine consumption and prenatal SHS exposure in women who do not smoke. There was a significant difference in maternal and infant hair nicotine measurement when comparing smoking, passive, and nonsmoking groups; however, infant hair nicotine was not able to discriminate between passive and nonexposure in women who do not smoke. Maternal hair also better reflected self-reported prenatal SHS exposure when compared with infant hair. These findings may be partly explained by the differences in maternal and infant hair development and structure.

Analysis of maternal and fetal/infant hair during or after pregnancy can pose significant challenges due to the vast interindividual differences in hair and various cutoff limits. Unique characteristics, such as hair texture, color, melanin concentration, ethnicity, growth rate, and rate of nicotine metabolism, may influence absorption. Evidence on the metabolic changes that influence nicotine/cotinine absorption during pregnancy point to increased nicotine clearance late in pregnancy (Koren, Blanchette, Lubetzky, & Kramer, 2008). Current research hypothesizes that ethnicity/race also influences nicotine metabolism via the CYP2A6 pathway (Derby et al., 2008). Previous studies have also reported decreased hair nicotine levels based on bleaching, dyeing, and degree of hair damage (Jurado, Kintz, Menendez, & Repetto, 1997; Pichini et al., 1997). Fetal hair produces vellus hair, a fine and silky hair with little pigmentation. Collection of infant hair within 48 hr of birth limited nicotine analysis to vellus hair. Little is known about individual differences in fetal hair collected from preterm infants (24–36 weeks at birth) compared with those who are more mature (≥ 37 weeks).

Use of infant hair nicotine analysis in determining exposure to direct maternal smoking is beneficial, although limited when calculating passive exposure. Infants of mothers who smoked had hair nicotine levels two and half times higher in comparison with infants of mothers who did not smoke. Generally, fetuses of mothers who smoke or who are exposed to SHS are subject to higher nicotine concentrations than their mothers (Hellstrom-Lindh & Nordberg, 2002). Fetal cotinine found in amniotic fluid and serum was reported as early as 7 weeks of gestation and also reported higher levels when compared with maternal urine cotinine (Jauniaux, Gulbis, Acharya, Thiry, & Rodeck, 1999). Our study did not support this assumption; conversely, we found lower hair nicotine levels in infants when compared with their mothers.

Hair nicotine analysis better reflects long-term exposure to nicotine and SHS over biological fluids. A limitation of serum, urine, or saliva cotinine versus hair cotinine or nicotine measurement is the relatively short half-life of cotinine in biological fluids (20 hr; Benowitz, 1999). Hair is one of the few biomarkers that provide a measure of chronic exposure to SHS in women who do not smoke (Jaakkola et al., 2001; Klein & Koren, 1999; Pichini et al., 2003). In a sample of young children, researchers reported that hair nicotine was a more precise measure of exposure than urine cotinine when compared with self-report (Al-Delaimy et al., 2002). Hair nicotine analysis used in this study (Mahoney & Al-Delaimy, 2001) was a valid tool for biomarker confirmation of smoking and exposure status in pregnant women and infants.

Historically, due to under/misreporting smoking status during pregnancy, biological confirmation of smoking status offers a more objective measure over self-report. Unexpectedly, only a 3% discrepancy existed between self-reported smoking status and urine cotinine confirmation. Collection of reported smoking behaviors after birth and during hospitalization resulted in nearly 95% of women reporting accurate smoking status. Webb et al. (2003) reported that as high as 70% of pregnant women had urine cotinine levels inconsistent with self-report. Urine cotinine levels of >80 ng/ml validated smoking status in 98% of pregnant women in the immediate postpartum period (Higgins et al., 2007; Koren et al., 2008) reported a strong correlation between the average number of cigarettes smoked per day in both nicotine and cotinine throughout each trimester. Urine cotinine levels in our study were not used to measure prenatal SHS exposure; however, previous reports using more complex urine cotinine analysis define passive smokers as <15 ng/ml (Jarvis, Tunstall-Pedoe, Feyerabend, Vesey, & Saloojee, 1987).

Social pressures regarding smoking during pregnancy and anxiety regarding birth outcomes may be lessened after birth. In our study, two women who answered “yes” to the question “*Do you currently smoke cigarettes?*” were misclassified as nonsmokers based on their urine cotinine. Because most hospitals are smoke free and women during labor are prohibited from smoking, collecting data within 24 hr of delivery in smoke-free facilities may limit accuracy in predicting smoking status. The mother’s knowledge of biomarker collection and the timing of data collection may have also influenced the accuracy of self-reported smoking behaviors. Studies reporting high deception rates primarily collect data on smoking status and consumption early in pregnancy via questionnaire or after delivery in the participant’s home (Bottorff et al., 2000; DeLorenze et al., 2002; Webb et al., 2003).

Level of maternal and infant hair nicotine significantly correlated with one another, and maternal hair was a more valid measure of prenatal SHS exposure and smoking status. Maternal hair was strongly correlated with all measured smoking behaviors, while infant hair only moderately correlated with the ordinal smoking variable and number of cigarettes smoked per day. The strongest relationship was found between MHN and the ordinal smoking variable. Mother–baby hair nicotine levels were also closely associated with urine cotinine levels.

Limitations

Interindividual hair differences and limited research on hair nicotine cutoff limits during pregnancy pose limitations while conducting hair nicotine analysis. Although MHN cutoff values for smoking/exposure status of pregnant women were not available at inception of the study, current research recommends prenatal hair nicotine levels of 0.2 ng/mg to distinguish active smokers from passive or unexposed (Florescu et al., 2007). Our cutoff value for MHN was 0.3 ng/ml and slightly higher than recommended.

Because quota sampling is a nonrandom sampling method, sampling error cannot be calculated. Although a sample size of 210 provided adequate power, there were small ethnic subsamples which could account for variations in hair nicotine content. Other sampling issues include the higher distribution of male infant and preterm births (PTBs). There is no clear explanation for the higher than expected number of males; however, high PTB rates in Kentucky may account for the 20% PTB rate. Over the past decade, the rate of PTB in the United States has steadily increased from 12.5% in 2004 to 12.8% in 2006 (Martin et al., 2009). Currently, Kentucky posts one of the highest PTB rates in the nation (15.2%). Furthermore, nearly one in four pregnant women in Kentucky smoke (CDC, 2004), a dominant risk factor for PTB.

Due to cost of laboratory cotinine analysis, validation of smoking status was based on a commercial urine assay and not analyzed in a licensed laboratory. Although not ideal, this assay is cost-effective and repeatedly correlates with more complex urine cotinine analysis (Bernert et al., 2005). Because most hospitals are smoke free and women are prohibited from smoking during labor, use of urine cotinine within 24 hr of birth likely led to misclassification of at least two participants.

Recommendations for future research

Timing of biomarker collection during the pregnancy, choice of SHS exposure sources, and biomarker feedback are important areas for future research. Studies have consistently demonstrated the need for a comprehensive account of exposure sites in order to gain an accurate reflection of exposure (Rebagliato et al., 1995). Limitations exist when pregnant women who do not smoke are only asked about "partner" smoking or "home" smoking. Measurement of self-reported prenatal SHS exposure should minimally be defined as: (a) number of household smokers and visitors; (b) number of hours of SHS exposure in home, vehicle, and work; and (c) average number of cigarettes smoked per day by others in the home. Careful consideration of the timing of biomarker collection is essential to identify accurately smoking status. Continued research using segmental hair nicotine analysis will help explain differences in nicotine metabolism throughout pregnancy.

Conclusion

Hair nicotine analysis is a potent measure of maternal and fetal exposure to direct maternal tobacco consumption and SHS exposure during/after pregnancy. Although mother–baby hair samples were moderately correlated, MHN was a more precise biomarker of self-reported prenatal SHS exposure than infant

hair. Infant hair nicotine was a reliable biomarker when comparing smoking status. Urine cotinine (NicAlert) was a cost-effective assay that strongly correlated with self-reported smoking status; however, it was not able to discriminate between passive and nonexposure. There continues to be variability in measurement of prenatal smoking and SHS exposure, possibly due to lack of clear operational and conceptual definitions of prenatal SHS exposure, variations in timing of biomarker collection, and/or variations in biomarker cutoffs and analysis. There appear to be lower deception rates for reported smoking status when maternal biomarkers are collected shortly after birth as compared with collection during pregnancy. Finally, it is important to collect long-term versus acute biomarkers of tobacco consumption in order to estimate accurately prenatal smoking and SHS exposure, as well as incidence of adverse birth outcomes.

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Declaration of Interests

None declared.

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