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SHORT REPORT

Intrauterine tobacco exposure may alter auditory brainstem responses in newborns

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Abstract

This study of tobacco exposure and auditory processes was conducted in a predominantly low-income population of 40 pregnant women and their newborns. Urinary cotinine concentrations and self-reported smoking status were obtained from the mother during the first prenatal care visit. Auditory brainstem-evoked responses (ABRs) were recorded in neonates to assess neuroelectrical activity of the auditory nerve following a sound stimulus. Infants of mothers with the highest cotinine concentrations (>1,000 ng/ml) responded at a rate that was four times greater (hazard ratio 4.1, 95% confidence interval 1.4–11.5) than infants of non-smoking mothers (cotinine ≤ 15 ng/ml). Associations with more moderate cotinine concentrations (>15–1,000 ng/ml) were not observed. Enhanced ABRs may disrupt auditory processes related to speech perception, negatively affecting reading and language development during childhood. The results suggest that tobacco exposure during pregnancy may impair auditory function.

Key words: *Tobacco, smoking, cotinine, auditory brainstem response, newborn*

Introduction

Maternal tobacco use has been consistently associated with harmful effects on fetal growth and development, but tobacco use during pregnancy remains prevalent in the United States and other countries (1). Growing evidence suggests that intrauterine tobacco exposure may negatively influence neurodevelopment leading to deficits in motor, sensory and cognitive function (2). The contribution of tobacco exposure to the impairment of auditory processes has received limited attention. Recent findings suggest that auditory function in infants may be altered by intrauterine exposure to tobacco smoke, as measured by retrospective reports of exposure collected after delivery (3). The purpose of this study was to investigate the association between intrauterine tobacco exposure and auditory processes in neonates, using an objective measure of tobacco exposure collected early in pregnancy.

Material and methods

The study was conducted within a cohort of 110 pregnant women from a predominantly lower socioeconomic status population who received prenatal care in the Women's Clinic in the Department of Obstetrics and Gynecology at the University of Oklahoma Medical Center in Oklahoma City, Oklahoma, USA. From February 2007 to June 2007, 363 women attended their first prenatal care visit for the current pregnancy. Eighty-three women (23%) were not eligible to participate due to maternal age <18 years or gestational age >22 weeks. Of the

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280 eligible women, 130 (46%) were approached for participation. Of those invited, 20 declined, resulting in a participation rate of 85% (110/130).

Maternal urine samples and interview data, including self-reported smoking status, number of cigarettes smoked per day and living with a smoker in the home, were collected at enrollment. Written informed consent was obtained from all study participants. The study was approved by the University of Oklahoma Health Sciences Center Institutional Review Board for human subjects research.

Cohort members were followed through delivery and a total of 78 live-births were identified. Eleven pregnancies resulted in spontaneous abortions. The remainder: reported moving out of area or transferring care to another facility ($n = 4$), did not return to the facility for care ($n = 16$), or had an implausible delivery date and was presumed to be a subsequent pregnancy immediately following the loss of the index pregnancy ($n = 1$).

Auditory brainstem-evoked responses (ABRs) were measured in both ears of the newborn by a licensed audiologist (E.S.) using Vivosonic Integrity™ (Toronto, Ontario, Canada) instrumentation. ABR testing was completed on 62 newborns. Reasons for not conducting ABR tests included admission to the neonatal intensive care unit ($n = 5$), hospital discharge before testing could be conducted ($n = 8$), early neonatal death ($n = 1$), refusal of hearing test ($n = 1$), and unfavorable testing conditions ($n = 1$). Testing conditions occasionally allowed for only one ear to be tested due to patient scheduling, baby's activity level or technical difficulties. These infants ($n = 4$) were excluded from the analyses along with one infant for whom date of delivery was not recorded and two infants tested more than seven days after delivery. When compared to the 55 with complete ABR measurements, the subgroup of live-births excluded for missing, incomplete or delayed ABR assessments ($n = 23$) had a somewhat higher proportion of self-reported smokers (30 vs. 25%).

ABR testing typically took place within the first two days of life and was conducted in the mother's hospital room. ABR waveforms are measures of neuroelectrical activity produced by the VIII cranial nerve and auditory brainstem in response to a sound stimulus. The ABR waveform is a measure of the amplitude of the signal (in microvoltage) plotted against post-stimulus time (in milliseconds). A standard ABR tracing at birth will contain three dominant peaks occurring within 10 milliseconds (ms) following the stimuli presentation. Wave V is selected for measurement in this study because it is the most prominent wave in newborns, representing the response of the upper brainstem. The signal latency for wave V represents the time (ms) for electrical activity to travel along the auditory nerve to the brainstem following the sound stimulus.

Electrodes were placed in a standard montage with the non-inverting electrode on the high center forehead (Fz = midline), the inverting electrode on the earlobe and the ground on the low forehead (Fpz). A probe placed in the infant's ear produced a click stimulus at 27.5 clicks per second. ABR parameters included the collection of a minimum of 600 equivalent sweeps of alternating polarity clicks for identification of responses at an intensity of 35 dB nHL (which is the intensity commonly used for newborn hearing screening in the USA). The equivalent sweeps are the Vivosonic representation of the signal to noise ratio for the Kalman-filtered ABR which gives more weight to the responses with less myoegenic artifact than those contaminated with artifact. The stimulus window was set to 20 ms. Band filters were set at 30–1,500 Hz. Impedance measures were below 5,000 μohms. Three runs of the responses were collected to assure reproducibility of the auditory responses. The waveform exhibiting the best morphology within replicated pairs was selected for measurement.

This investigation was supplemental to an original study of environmental exposures during pregnancy. Thus, analyses of maternal urinary cotinine concentrations were conducted for birth cohort members who had a sufficient quantity of urine remaining in –20°C freezer storage ($n = 80$) at the time of this ancillary study. Urine samples (2 ml) were shipped to Labstat International ULC (www.labstat.com) in December 2007 for cotinine estimation using high resolution capillary-column gas chromatography with split/splitless injection, a fused silica capillary column and detection by a thermionic specific detector. Control samples with low and high cotinine yields were analyzed in conjunction with test samples to ensure that the observed yields were within three standard deviations of the established average of all previous yields. For statistical analyses, concentrations below the limit of detection (LOD = 1 ng/ml) were assigned values equal to the LOD divided by the square root of two (4). Similarly, concentrations between the LOD and the limit of quantification (LOQ = 3.4 ng/ml) were assigned values equal to the LOQ divided by the square root of two. Creatinine (mg/dl) was also assessed as a measure of urine concentration.

Statistical analyses

A total of 40 mother–baby pairs had complete maternal cotinine and neonatal ABR measurements available for analysis. Self-reported maternal smoking

status was available for all subjects with complete ABR testing; thus, secondary analyses of mothers' self-reported smoking data were conducted among these 55 neonates. When characteristics of the two samples were compared, the study group with urinary cotinine measurements (n = 40) had a somewhat smaller proportion of primiparous women compared to the group with self-reported smoking data (n = 55) (22 vs. 28%, respectively) and a slightly greater proportion enrolled ≤ 14 weeks of gestation (73 vs. 68%). All other characteristics, including self-reported smoking, were distributed similarly across the two groups.

Urinary cotinine concentrations were categorized as ≤15 (reference), > 15 to ≤1,000, and > 1,000 ng/ml, with the reference category selected to distinguish active smokers from non-smokers (5) and the upper category selected to identify the heaviest smokers. Number of cigarettes smoked per day was categorized as 0, 1–9, and ≥10. Self-reported secondary smoke exposure was assessed as living with anyone who smoked cigarettes or other tobacco products regularly in the home (yes/no). A kappa statistic was calculated to estimate percent agreement beyond chance when comparing smoking status measured by urinary cotinine > 15 ng/ml and self-report. According to the criteria published by Altman (6), the kappa statistic was interpreted as: < 0.20 poor agreement, 0.21–0.40 fair agreement, 0.41–0.60 moderate agreement, 0.61–0.80 good agreement, and 0.81–1.00 very good agreement.

Gestational age at delivery and infant characteristics were obtained from the medical record. Clinical estimates of gestational age were based on date of last menstrual period as confirmed by ultrasound. When gestational age at delivery was missing from the record (n = 4), an estimate was calculated using weeks of gestation on the date of recruitment and the recorded date of delivery. Because maturational changes in ABR wave V latency occur with advancing gestation and continue after delivery through the first one to two years of life (7), postmenstrual age at testing (gestational age + chronological age) was also considered.

Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazards models. The HR compares the rate of response in the exposed infants to the rate of response in the unexposed. Separate models were estimated for the latency response tested in each ear and for the shortest latency response from either ear. Infants without a detectable wave V response within the stimulus window were censored at 20 ms. Confounding was assessed by evaluating the change (> 10%) in the HR for tobacco exposure when controlling for the factor in the model. Due to the small sample size, each covariate was assessed independently. Maternal and infant characteristics evaluated as potential confounders included postmenstrual age at testing, maternal age, maternal race, infant sex, education, household income, birth weight and creatinine concentrations. Only maternal age met the criteria for confounding and all models are adjusted for this factor.

Several sub-analyses were conducted after excluding infants who: (1) tested more than two days after delivery (n = 3), (2) delivered before 259 days of gestation (n = 6), (3) tested at postmenstrual age < 259 days (n = 5), (4) had a one minute Apgar score less than seven or the Apgar score was missing (n = 3), or (5) had a birth weight < 2,500 grams or missing (n = 4). The findings remained consistent in all sub-analyses. The magnitude of the point estimate generally increased for the most highly exposed group, but with wider CI. Results are reported for analyses including all 40 infants.

Results

The characteristics of the study population are described in Table I. This predominantly low income population is racially/ethnically diverse, with close to half under the age of 23 (45%). Most women had their first prenatal care visit during the first trimester of pregnancy (73%). Mean gestational age at enrollment was 12 weeks (range 4–22).

Ten women (25%) reported being a current smoker at the time of enrollment. Eighteen (45%) reported living with a smoker, of whom 10 did not report smoking themselves. There was only moderate agreement between cotinine concentrations and self-reported smoking status (kappa = 0.58, 95% CI 0.34–0.82). No self-reported smokers had cotinine concentrations < 15 ng/ml. However, of the 30 self-reported non-smokers, eight (27%) had cotinine concentrations > 15 ng/ml (four below 27 ng/ml, two between 100 and 700 ng/ml, and two above 2,000 ng/ml). Only two of these women reported living with a smoker, and they were both in the < 27 ng/ml category for cotinine. Among the seven women with urinary cotinine concentrations above 1,000 ng/ml, three reported smoking an average of 10 cigarettes per day and living with a smoker, two reported smoking five cigarettes per day on average and not living with a smoker, and two denied smoking themselves or living with a smoker.

Most babies (93%) received ABR testing within the two days following delivery. For six (15%) infants, a wave V response was not detected in at least one ear within the stimulus window. This is greater than the

Table 1. Characteristics of the 40 study participants with urinary cotinine measures and auditory brainstem-evoked response test results.

	N	%[a]
Maternal age (years)		
18–22	18	45
23–31	22	55
Mean (SD)	23.2 (3.3)	
Race/ethnicity		
Non-Hispanic White	15	37
Non-Hispanic Black	14	34
Hispanic	9	23
American Indian	1	2
Asian	1	2
Education		
≤High school	22	55
>High school	18	45
Income		
<$10,000	20	50
$10,000–$29,999	16	40
≥$30,000	4	10
Gestational age at enrollment (weeks)		
4–14	29	73
15–22	11	27
Mean (SD)	12.0 (4.2)	
Self-reported smoker		
Yes	10	25
No	30	75
Gravidity		
1	9	23
2	15	38
≥3	16	40
Birth weight (g)		
<2,500	3	8
≥2,500	36	90
Missing	1	3
Mean (SD)	3,356 (488)	
Apgar (1 min)		
≤6	2	5
≥7	37	93
Missing	1	3

[a]Percentage totals may not add to 100% due to rounding.

2% of infants in the general United States population who are reported by the Centers of Disease Control and Prevention to fail newborn hearing screening tests (8), which is often conducted using automated ABR testing. This difference may be the result of our small sample size or the characteristics of the underserved population, which may represent infants at greater risk of altered ABR responses. Only one of the six without a detectable wave V response was born to a self-reported smoker.

Table 2 displays the HRs and 95% CIs for the association between tobacco smoke exposure and wave V latency response at 35 dB. When considering the minimum latency response tested in either ear, infants of mothers with the highest cotinine concentrations (>1,000 ng/ml) responded at a rate that was four times greater (HR 4.1, 95% CI 1.4–11.5) than infants of non-smoking mothers (cotinine ≤ 15 ng/ml). Associations with more moderate cotinine concentrations (>15–1,000 ng/ml) were not observed. This pattern of association was consistent when the ABR results from each ear were analyzed separately. When tobacco exposure was assessed by self-report, smoking 10 or more cigarettes per day was associated with a similar magnitude of effect when considering responses obtained from either ear or the left ear alone. An increased rate of response among self-reported smokers was not observed in the right ear. No associations were observed for self-reported secondary smoke exposure when assessed independently or in combination with self-reported smoking.

Discussion

The primary limitations of this study are the small sample size and the single exposure assessment to quantify tobacco exposure for the entire pregnancy. Cotinine concentrations reflect total exposure to nicotine from active and passive exposure to tobacco smoke. The use of urinary cotinine as a biomarker for tobacco exposure avoids the pitfalls of recall error or deception that may occur with self-report. These measures, however, reflect only recent exposures given the relatively short half-life of cotinine. Studies have shown that cotinine levels among smokers tend to remain relatively constant over time (9). However, many women who smoke tend to reduce the amount smoked while pregnant, if they do not quit. This study was limited to assessing maternal cotinine levels early in pregnancy and no measurements of changing smoking habits throughout pregnancy were available for analysis. Measurement error would be introduced if exposure assessed at the enrollment visit does not reflect smoking patterns maintained during the critical window of susceptibility.

These results suggest that sensory processing of auditory stimuli may be disrupted by intrauterine exposure to tobacco smoke. Our findings are

Table 2. Hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between tobacco smoke exposure and auditory brainstem-response latency at 35 dB nHL.

		Right ear		Left ear		Fastest response in either ear	
	n	HR[a]	95% CI	HR[a]	95% CI	HR[a]	95% CI
Cotinine (ng/ml)							
≤15	22	1.0	–	1.0	–	1.0	–
>15 to ≤1,000	11	1.5	0.7–3.4	0.9	0.4–2.1	0.8	0.4–1.9
>1,000	7	4.9	1.7–14.1	4.4	1.5–12.6	4.1	1.4–11.5
Self-reported number of cigarettes smoked per day							
0	41	1.0	–	1.0	–	1.0	–
1–9	9	1.5	0.7–3.1	0.8	0.4–1.7	0.7	0.3–1.6
≥10	5	1.7	0.6–4.5	3.5	1.2–9.9	4.5	1.5–13.2

[a]Controlling for maternal age.

consistent with previous reports of dysregulation of auditory processing following prenatal nicotine exposure. A recent study by Kable et al. reported that smoking before and during pregnancy was associated with decreased ABR wave V latencies in infants examined at six months of age (3). In an animal model, rats subjected to chronic nicotine exposure during development exhibited reduced latencies in adulthood (10). Evidence for the underlying mechanism suggests diminished function of the nicotinic acetylcholine receptors in the cerebral cortex (10).

Although prolonged ABR latencies are used clinically to indicate neuropathology and hearing deficits, rapid ABR latencies have been associated with learning disabilities and language impairment in school-age children (11,12). It is hypothesized that ABRs that occur too quickly may disrupt auditory processes related to the perception of speech, adversely affecting reading and language development during childhood (3). The results of our study suggest the adverse effects of smoking during pregnancy may include impaired auditory function. The stimulatory effects of nicotine exposure during critical stages of neurodevelopment warrant further investigation.

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