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Prenatal Smoking Exposure and Neonatal DNA Damage in Relation to Birth Outcomes

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ABSTRACT: This study investigated whether mothers with prenatal environmental tobacco smoke (ETS) exposure increased the newborn genetic damage and adverse birth outcomes. Study participants were women receiving prenatal care at three hospitals in Central Taiwan and their newborns. Participants were divided into two groups (non-smokers and ETS-exposed non-smokers) based on maternal ETS-exposed status. Comet assay were performed for cord blood samples. Infants born to mothers with prenatal ETS exposure had the highest mean cord blood DNA damage score (69.7 ± 42.3) and poorer birth outcomes. No negative fetal growth effects appeared among newborns with low DNA damage levels. Among newborns with high DNA damage levels (comet scores >50), those born to prenatal ETS exposure had an average reduction of 252.7 g in birth weight, 1.10 cm shorter in length and a 0.92-cm decrease in head circumference, compared to newborns with no smoking exposure. This study shows that the DNA damage scores can be used as an effect-modifier on the relationships between ETS exposure and adverse birth outcome. The association appears more apparent for the ETS exposure in relation with more severe DNA damage. **(*Pediatr Res* 64: 131–134, 2008)**

Studies have suggested adverse reproductive outcomes among newborns in relation to maternal cigarette smoking and environmental tobacco smoke (ETS) exposure during pregnancy. Most of these studies are based on self-reported smoking status, and serum or urinary cotinine levels as the exposure measures (1–6). Some studies failed to find a significant association (7–11). The discrepancy could be explained by the variability of study populations, sample size, study design, and individual susceptibility (12,13). Other markers linking the association between smoking and/or ETS and pregnant outcomes deserve exploration.

Cigarette smoke contains more than 4000 chemicals (14), including carcinogens such as polycyclic aromatic hydrocarbons (PAHs), arylmines, N-nitrosamines (15,16), and aromatic amines. These compounds can cross the placenta and experimental animal studies have indicated that the fetus and newborn are more susceptible to carcinogens than adults (17–19). A study of white population showed that there is an increased susceptibility to DNA damage from PAHs and the diminished ability to clear ETS components for the fetus (20). de la Chica *et al.* found that maternal smoking exposure increase structural chromosomal abnormalities and chromosomal lesions in fetus (21). However, the effect of genotoxicity determined by comet assay for infants with prenatal smoking exposure has not been well documented. The comet assay is a sensitive technique allowing the detection of DNA damage at the single cell level. This assay is also effective in detecting DNA damage induced by tobacco smoke toxicants in white blood cells (22).

The smoking prevalence among women in Taiwan has recently increased to 5.95%, and as many as 42.4% of women are daily exposed to ETS (23). Another studies in Taiwan showed that 58% of pregnant women and 57% of never-smoked women in the reproductive age had exposed to ETS either at work or at home (24,25). Our previous investigation indicated that the mean DNA damage level among women with ETS-exposure at work and/or at home was significantly higher than that among non-exposed women (24,26). We hypothesized that the scored comet can be used as the biomarker linking the relationship between the prenatal tobacco smoke exposure and the adverse birth outcomes. This study is the first report using the comet assay to measure neonatal DNA damage and the risk of giving adverse birth outcomes associated with prenatal maternal ETS exposure.

MATERIALS AND METHODS

Participants, data collection, and sampling. All pregnant women receiving prenatal care between August 2003 and October 2004 at three hospitals in the Central Taiwan were invited to participate in this study. Three hundred eighty-three pregnant women agreed to participate during the third trimester of gestation, and most delivered live singletons. A questionnaire was used to collect information on demographics, smoking status, medical history, and lifestyle habits for each participant. Mothers were divided into three smoking status groups based on their responses to the following questions in the questionnaire: "Have you ever been a smoker? ", "Does your spouse smoke?" "Are there any smokers in your household?", and "Can you smell people smoking in your workplace?" A smoker in this study was defined as a mother who currently smoked or had quit smoking after conception. Subjects who had never smoked were assigned to the non-smoker group, and those who did not smoke but were exposed to ETS were assigned to the ETS-exposed group.

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Abbreviations: ETS, environmental tobacco smoke; **PAHs,** polycyclic aromatic hydrocarbons

However, a total of 24 women were grouped as smokers, excluded because of small sample size.

There were 184 ETS-exposed mothers who had either a smoking partner or could smell people smoking at work. The remaining 175 women were non-smokers. Gestation age (GA) and neonatal birth outcome data (*e.g.*, birth weight, length, head and chest circumference, duration of gestation) were extracted from the medical records. The GA was estimated based on the last menstrual period reported to the physician in all three hospitals.

Blood sampling. Umbilical cord blood samples were obtained from 306 newborns at the deliver. Among them, 93 cord-blood samples had hemolysis phenomenon or had not sufficient volume for the comet assay. Fifteen smokers were excluded because the sample size was too small. In addition to umbilical cord blood specimen, 3–5 mL of whole blood was drawn from each mother. For each umbilical cord, 3 mL was kept in heparin tubes for the comet assay. All pregnant women gave informed consents to participate in the study and the study was approved by the University Human Subjects Committee.

Comet assay. The comet assay was performed on cord blood samples available for 198 infants born to 104 ETS-exposed participants and 94 non-smokers. Lymphocytes were carefully isolated from the blood using a Histopaque 1077 (Sigma Chemical Co., St. Louis, MO). The buffer coat containing lymphocytes was washed three times with phosphate buffered saline (PBS). Lymphocytes were evaluated for viability before the experiment with trypan blue dye and counted in a Neubauer chamber. Single-cell gel electrophoresis (comet assay) was conducted with the method adapted from Wu *et al.* (26) and McNamee *et al.* (27). The lymphocyte suspension containing about 5×10^5 cells/mL was placed on microscopic slides. Cells were stained with 40 μL propidium iodide (PI) solution and viewed under a fluorescence microscope (Olympus BX51, Tokyo, Japan), equipped with an excitation filter BP520–550 and a barrier filter BA580-IF, and examined at $\times$100 magnification. The microscope was connected to a high-sensitivity CCD video camera and a computer with an image analysis system.

The observed extent of DNA damage per cell was calculated by measuring 100 nuclei per participant from two slides. All slides were scored by one reader who was blind to the smoking status of the participants. According to Anderson *et al.* (28), DNA damage is expressed as a percentage of total fluorescence migrated to the tail in each nucleus (DNA % in tail), and quantified by visually classifying cells into five categories: no damage (<5%), low-level damage (5–20%), medium-level damage (20–40%), high-level damage (40–95%), and complete damage (>95%). The DNA damage score for each subject was calculated as: (no damage $\times$ 0) + (low damage $\times$ 1) + (medium damage $\times$ 2) + (high damage $\times$ 3) + (complete damage $\times$ 4) for each nucleus.

Statistical analyses. Data analyses were performed using SAS statistical software for Windows version 9.1 (SAS institute, Cary, NC). Newborns with gross birth defects and twins were excluded from data analysis. We compared the characteristics of pregnant women and newborns by maternal smoking status (non-smokers and ETS exposed non-smokers). We did not distinguish ETS by spouse from other members because there was no significant difference in mean DNA damage scores between their two types of ETS exposure. Maternal age at the pregnancy, employment, education and drinking, and the newborn sex, gestational age, and body weight, length, head circumference and chest circumference at the birth were compared. The average DNA damage scores obtained from comet assay for cord blood specimens were compared among newborn by maternal ETS-exposed status. The LOESS function (offered by SAS software) identified a specific DNA damage score as the cutoff point for further stratified analysis. Linear regression analysis was used to estimate the fetal growth measures in relationship with maternal ETS-exposed status. This analysis was conducted by stratifying newborns into low and high DNA damage levels using the cutoff score determined by the LOESS function. In the linear regression analyses, maternal age and education, gestational age, employment status, and alcohol drinking were also considered as covariates for adjustment.

RESULTS

Near half the mothers were exposed to ETS who had higher employment rate and received less education (Table 1). Infants born to never smoking mothers had higher average birth weight than infants of ETS-exposed groups but not significant. There were also no differences in average lengths, head circumferences, and chest circumferences between these two groups of neonates.

Neonates of ETS-exposed mothers had the highest average DNA damage score (69.7) followed by those born to non-smokers (54.0) (Table 2). The difference between two groups was statistically significant. The LOESS regression analysis showed that the birth weights had a tendency to decrease as the level of DNA damage scores increased. This inverse relationship was even more pronounced when DNA damage scores were greater than 50. The birth lengths increased slightly at first for neonates with DNA damage scores less than 50 and gradually decreased as the DNA damage scores continued to increase. The DNA damage scores had no significant associations with both head and chest circumferences (data not shown).

Based on the LOESS function, we divided neonates into two strata, low-level DNA damage ($\leq$50) group and high-level DNA damage (>50) group, for further linear regression analyses (Table 3). Comparing with non-smoking mothers and ETS-exposed mothers appeared at higher risk of having the adverse fetal growth among their newborns with high-level DNA damage. In the low-level DNA damage stratum, no significant adverse birth effects were found. After adjusting for the covariates in the regression model, the mean birth weights of neonates in the ETS-exposed group was significantly lower than that in the non-smoking group (lowered by 252.7 g, $p = 0.005$). Neonates in the ETS-exposed group also had a shorter mean body length by 1.10 cm ($p = 0.021$),

Table 1. *Characteristics of mothers and newborns compared by maternal exposure status*

	Nonsmoking	ETS-exposed nonsmoking	p*
Mothers, *n* (%)	175 (48.8)	184 (51.3)	0.002
Age (y) (SD)	30.9 (4.0)	29.5 (4.8)	0.817
BMI (kg/cm^2) (SD)	21.2 (3.0)	21.3 (2.9)	0.080
Gestational age (wk) (SD)	39.1 (1.3)	39.4 (1.2)	
Employment, *n* (%)	74 (43.0)	48 (26.8)	0.001
No	98 (57.0)	131 (73.2)	
Yes			
Education (y), *n* (%)	5 (2.9)	13 (7.1)	<0.001
<9	109 (62.3)	140 (76.1)	
10–12	61 (34.9)	31 (16.9)	
>13			
Alcohol consumption, *n* (%)	171 (98.3)	175 (96.7)	0.341
Never	3 (1.7)	6 (3.3)	
Currently or previously			
Newborns			
Sex, *n* (%)			0.112
Female	84 (48.6)	71 (40.1)	
Male	89 (51.5)	106 (59.9)	
Birth weight (g) (SD)	3206.1 (405.0)	3172.4 (392.2)	0.424
Length (cm) (SD)	51.0 (2.0)	50.8 (2.2)	0.273
Head circumference (cm) (SD)	33.5 (1.6)	33.5 (1.6)	0.768
Chest circumference (cm) (SD)	32.8 (1.7)	32.9 (1.7)	0.718

* Comparison of the characteristics of mothers and newborns between non-smoking group and ETS-exposed nonsmoking by *t*-test or χ^2 test.

Table 2. *Comparison of average cord blood DNA damage score by maternal exposure status*

	DNA damage score			
	n	Mean ± SD	Range	p
Nonsmoking	94	54.0 ± 33.8	7.02–179.5	0.004
ETS-exposed nonsmokers	104	69.7 ± 42.3	7.38–181.2	

Table 3. *Regression analysis for fetal growth measures in newborns associated with maternal exposure status stratified by cord blood neonatal DNA damage score*

DNA damage score*	*n*	Mean ± SD	Model 1†			Model 2†		
			β	SE	p	β	SE	p
			Birth weight (g)					
Low-level damage								
Non-smoking	48	3144.5 ± 396.7	Referent	—	—	Referent	—	—
ETS-exposed non-smokers	40	3147.2 ± 390.0	−0.8	86.2	0.992	78.5	91.4	0.393
High-level damage								
Non-smoking	46	3275.6 ± 414.2	Referent	—	—	Referent	—	—
ETS-exposed non-smokers	64	3108.8 ± 412.1	−166.8	79.8	0.039	−252.7	87.1	0.005
			Birth length (cm)					
Low-level damage								
Non-smoking	48	50.8 ± 1.9	Referent	—	—	Referent	—	—
ETS-exposed non-smokers	39[c]	50.4 ± 2.2	−0.44	0.44	0.318	−0.37	0.47	0.442
High-level damage								
Non-smoking	46	51.4 ± 2.0	Referent	—	—	Referent	—	—
ETS-exposed non-smokers	64	50.5 ± 2.3	−0.95	0.42	0.025	−1.10	0.47	0.021
			Head circumference (cm)					
Low-level damage								
Non-smoking	47[c]	33.2 ± 1.6	Referent	—	—	Referent	—	—
ETS-exposed non-smokers	40	33.4 ± 1.8	0.17	0.37	0.652	0.39	0.41	0.337
High-level damage								
Non-smoking	46	34.0 ± 1.6	Referent	—	—	Referent	—	—
ETS-exposed non-smokers	64	33.1 ± 1.6	−0.87	0.30	0.005	−0.92	0.36	0.013
			Chest circumference (cm)					
Low-level damage								
Non-smoking	48	32.5 ± 1.4	Referent	—	—	Referent	—	—
ETS-exposed non-smokers	40	32.8 ± 1.8	0.36	0.34	0.296	0.57	0.38	0.141
High-level damage								
Non-smoking	46	33.1 ± 1.7	Referent	—	—	Referent	—	—
ETS-exposed non-smokers	64	32.7 ± 1.7	−0.47	0.33	0.158	−0.70	0.38	0.073

* DNA damage score was divided into low-level (≤50) and high-level (>50) damage.

† Model 1 was a simple linear regression; Model 2 adjusted for maternal age, gestational age, BMI, occupation, education level and alcohol consumption.

smaller head circumference by 0.92 cm ($p = 0.013$), and chest circumference by 0.70 cm ($p = 0.073$), compared with the nonsmoking group.

DISCUSSION

Previous studies on the relationship between prenatal smoking status and birth outcomes have focused on newborn birth weight, birth length, and head circumference. Our study found that neonates with the antenatal ETS-exposed group had an average reduction of 33.7 g in birth weight, compared with non-smoking groups. Although these crude birth weight means were not statistically significant, they agreed with the trend of previous research (29).

After adjusting for potential confounders, our study found that neonates with the antenatal exposure to ETS had an average reduction of 252.7 g in birth weight, a 1.10 cm shorter in birth length, and a 0.92 cm smaller in head circumference, compared with non-smoking groups. In a notable study, Goel *et al*. found that infants born to mothers exposed to ETS had a mean birth weight of 362 g less than infants born to non-smoking mothers (30). Lazzaroni *et al.* have also demonstrated a mean birth weight reduction of 123.7 g for the infants with antenatal passive smoking exposure. A mean reduction of 51.4 g in birth weight and a 0.3-cm decrease in birth length were found in the 11 Italian cities study for ETS exposure; the mean birth head circumference of infants born to non-smoking mothers was found to be 0.1–0.2 cm ($p < 0.10$) wider than that of both passive smoking and ETS exposures (31).

Research of the harmful effect of ETS-exposed on the fetus using neonatal DNA damage as an indicator is limited. This study is the first using the comet assay to investigate the newborn DNA damage and its relationship to antenatal smoking exposure and birth outcomes. The comet assay is a well-established simple technique to determine genotoxicity. It has the advantage of time and effort saving compared with methods to assay sister chromatid exchange SCE and frequency of chromosomal aberrations. This assay has been widely used to detect DNA damage in human lymphocytes caused by tobacco and chemicals. Previous studies revealed that occupational exposure to wood dust, lead, and industrial vinyl chloride monomer increased the DNA damage levels in white blood cells of workers (32–34). Our study used the comet assay to assess the DNA damage levels in umbilical cord blood. The results showed increased DNA damage levels in neonates with antenatal ETS exposure. These results are consistent with the results found by examining SCE or frequency of chromosomal aberrations (21,35,36).

One of the World Trade Center (WTC) disaster studies has examined the birth outcome effect of PAHs adducts in maternal and umbilical blood from non-smoking mothers (37). No significant fetal growth effect of either prenatal ETS exposure or PAH-DNA adducts was found. But PAH-DNA adducts in

combination with the ETS exposure were associated with decreased newborn birth weight and head circumference. In our study, the fetal growth effects associated with ETS exposure at low DNA damage levels were less impressive. The LOESS function analyses appeared that the prenatal smoking exposure effect became more apparent when the comet scores increased beyond 50. This was why we conducted the multivariate regression analyses by stratifying DNA damage into low-level and high-level using the score of 50 as the cutoff. The low-level DNA damage association resembles the PAH-DNA adducts association in the WTC study. The high-level DNA damage association resembles PAH-DNA adducts in combination with the ETS exposure with a greater impact. The negative associations with cigarette smoke exposure become significant at high-level DNA damage, particularly of prenatal ETS exposure with higher DNA damage scores. The newborn weight, length, and head circumference were significantly reduced at higher DNA damage levels. This finding may reflect that the ETS exposure has a greater impact than nonsmoking group on fetus DNA damage. Although, this study was limited with a relatively small sample size and inability to examine other adverse birth outcomes such as fetal growth restriction, preterm birth, low birth weight.

In conclusion, our study found that the DNA damage scores could be used as an effect-modifier on the relationships between ETS exposure and adverse birth outcome. The DNA damage configuration associated with the intake of smoke components could be related to fetal development. This finding warrants additional studies for other types of health effect in addition to reproductive health. The findings in the fetal growth variation associated with the combination of maternal smoking status and DNA damage imply the importance of prenatal ETS exposure prevention.

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