Impact of Prenatal Tobacco Smoke Exposure, as Measured by Midgestation Serum Cotinine Levels, on Fetal Biometry and Umbilical Flow Velocity Waveforms

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ABSTRACT

The aim of this prospective cohort study was to evaluate the impact of tobacco smoke exposure, measured by maternal serum concentration of cotinine, on fetal midgestation biometric parameters and umbilical artery (UA) qualitative blood flow indices. The study population consisted of 114 healthy women in 20 to 24 weeks gestation who were recruited from the patients of two antenatal care units in Lodz, Poland. Significant negative correlation was found between fetal biparietal diameter (BPD) and serum cotinine concentration. Serum cotinine positively correlated with all blood flow indices under study (systolic/diastolic index [S/D], resistance index, and pulsatility index) after controlling for gestational age, gender, and femur length. The midgestation UA S/D ratio > 3 was found to be a significant risk factor for decreased birthweight. Tobacco smoke exposure is a significant factor inducing increased resistance of umbilical blood flow as measured in 20 to 24 weeks gestation. This could be one of the main mechanisms leading to decreased birthweight observed among infants with prenatal exposure to tobacco smoke.

KEYWORDS: Environmental tobacco smoke exposure, cotinine, uteroplacental circulation

The association between maternal cigarette smoking during pregnancy and the elevated risk for low birthweight, preterm delivery (PD), and intrauterine growth restriction has been well documented in many studies.[1–3] According to Kyrklund-Blomberg and Cnattingius,[2] there is a dose-related impact of smoking on the risk of preterm delivery. The highest impact refers to the risk of spontaneous very preterm births among women smoking at least 10 cigarettes a day (odds ratio, 1.7).

The sidestream smoke (SS), the main constituent of environmental tobacco smoke (ETS), contains similar constituents as does the cigarette smoke inhaled by active smokers, namely tar, nicotine, carbon mono- and dioxide, and benzo(a)pyrene. Moreover, the concentrations of the chemicals found in SS may (even by several times) exceed those in the smoke from tobacco combustion (mainstream smoke).[4–8] Many of these substances are known to pass through the placental barrier.[9,10]

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Given that the chemical composition of ETS differs quantitatively from the composition of the mainstream smoke, a question arises whether a nonsmoking pregnant woman exposed to ETS toxicants could also be at a higher risk for similar adverse effects on fetal health as is the smoking woman.

The impact of exposure to ETS (passive smoking) on pregnancy outcome has also been investigated in several studies, but the results are often controversial.[11–17] An increased risk was found for a small for gestational age (SGA) infant[12,14] and low birthweight (LBW) in term deliveries.[16] However, negative results[11,17] or borderline excess risk of LBW were also reported.[18] A relationship between ETS exposure and the rate of PD was postulated by Ahlborg et al[11] and confirmed for women with the highest levels of ETS exposure in comprehensive studies of pregnant women from central Poland[15] and the Czech Republic.[13] The contrasting results might be explained by the differences in the methodology used for ETS exposure assessment (questionnaire versus biomarkers) as well as different study populations, gestational age at the time of ETS exposure assessment, and definitions of the examined endpoints. Klebanoff et al[19] showed that pregnant women accurately reported whether they smoked, but the serum concentration of cotinine, the major metabolite of nicotine, was a better measure of the actual tobacco smoke exposure.

Hayde et al[20] observed that maternal cotinine levels correlated with maternal and fetal carboxyhemoglobin (COHb) levels, which suggests that maternal cotinine measurements may be interpreted as the surrogates of fetal COHb.

Few studies addressed the issue of the impact of ETS exposure on fetal biometry and placental vascular resistance.[21] The mechanism underlying the adverse effect of passive smoking on the course and outcome of pregnancy might be complex. One may presume that a long-term exposure to tobacco smoke constituents, even at low doses, may lead to an impaired fetomaternal blood flow circulation.[22] The blood flow velocity changes may be mediated by nicotine, carbon monoxide, catecholamines, or some other factors related to smoking. It has been speculated that smoking causes an increase in the placental vascular resistance both in humans and animals.[21,23,24]

According to Newnham et al,[25] to explain the association between tobacco smoke exposure during pregnancy and fetal growth, there is a need for studies that would be based on prenatal ultrasound measurements rather than the findings of the infant's examination after birth.

The main aim of this prospective cohort study was to evaluate the impact of tobacco smoke exposure, measured by serum concentration of cotinine, on fetal midgestation biometric parameters and umbilical artery (UA) qualitative blood flow indices.

MATERIALS AND METHODS

Subjects

The study population comprised a group of 129 healthy women in 20 to 24 weeks gestation who were enrolled consecutively at two antenatal care units in Lodz, central Poland, over a 1-year period. Those two units served mostly an indigent population characterized by a high prevalence of smoking and ETS exposure, compared with communities from other regions of central Poland.[11] Only the singleton pregnancies were qualified for inclusion in the survey. Women with chronic diseases diagnosed during the first visit were not considered. A standard questionnaire covering medical, socioeconomic, demographic, and constitutional aspects as well as tobacco smoking, including ETS exposure, was administered to every subject and verified based on medical records. Routine ultrasound examination of fetal biometry and Doppler umbilical blood flow assessment was performed along with serum cotinine determination for assessment of tobacco smoke exposure. For five subjects, complete medical records of the newborns were not available. Ten women refused to have serum cotinine measurement ($n = 8$) or Doppler ultrasound examination ($n = 2$). This prospective cohort study was approved by the Ethical Committee of the Medical University of Lodz, Poland (Decision No. RNN/212/97). Each participant provided a written consent for participation in the study. After ultrasound examination, women who reported smoking or ETS exposure were strongly advised to stop smoking and avoid ETS exposure.

Ultrasound Examination

Ultrasound biometric measurements of fetal biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) were performed between 20 and 24 weeks of gestation. Each ultrasound measurement was made three times and the mean values were considered for additional analysis. Apart from routine ultrasound, Doppler flow velocimetry in the UA was conducted at the same time. Fetal Doppler waveform examination was made using pulsed Doppler ultrasound after identifying the appropriate vessel by color flow imaging. For all Doppler studies, a 3.5-MHz transducer was used (Hitachi EUB 315C). Recordings were made from the UA by placing the sample volume in the lumen of the artery, away from the placental and fetal cord insertion. Once the satisfactory waveform was imaged, the image was frozen and the blood flow indices (systolic/diastolic index [S/D], resistance index [RI], and pulsatility index [PI]) were calculated.

Serum Cotinine Measurement

Serum cotinine concentration was determined by gas chromatography with mass spectrometry detector to assess ETS exposure during the morning hours of the same day (blood collection at 1200 to 1300 hours). The limit of detection was 0.16 ng/mL, whereas the limit of quantification was 1.25 ng/mL. The half-life of cotinine averages ~17 hours. With intermittent nicotine exposure, which occurs with cigarette smoking or ETS exposure, cotinine levels remain relatively constant throughout the day and remain at near steady-state values.[26]

In our study, ETS exposure (passive smoking) was diagnosed when the level of serum cotinine ranged between 2 and 14 ng/mL. The subjects regarded as nonsmokers with no ETS exposure had to have serum cotinine levels lower than 2 ng/mL. The subjects were classified as biochemical (active) smokers when their cotinine levels exceeded 14 ng/mL in blood serum.[27]

Statistical Analysis

The differences between the mean values of quantitative variables were analyzed using linear regression. Multiple linear regressions for BPD, AC, and FL included gestational age at the time of ultrasound examination and fetal gender, whereas those for S/D, RI, and PI also the fetal femur length (FL), were an indicator of fetal size. On the basis of the multiple linear regressions models, the adjusted values of BPD, AC, and FL were calculated for median values in each of the cotinine groups. The differences in the distribution of qualitative variables were evaluated by χ^2 test with Yates correction when necessary. The serum cotinine concentrations were transformed into logarithmic values because they did not meet the criterion of normal distribution.

RESULTS

Study Population Characteristics

The mean age of the examined group was 26.3 years. In the study population, 21% of women were unmarried and 16.1% had primary education. The proportion of women with these characteristics increased with the level of serum cotinine; however, the differences were not statistically significant.

Most of the study group (51.8%) included primiparous women and the mean gestational age was 22.8 weeks. Women with the highest cotinine levels were most frequently primiparous and at a higher gestational age (nonsignificant differences). The proportion of male and female gender of the fetus was well balanced in all of the examined groups, with a slight excess of the male gender in the group with a lower cotinine level (Table 1). As expected, the mean birthweight and gestational age of newborns at delivery was the lowest in the group with highest cotinine levels. The largest proportion of SGA infants (i.e., infants with birthweight below the 10th percentile for a given gestational age, using the national statistics) was found in the group with the highest cotinine levels (9.1%), which, however, was not statistically different from other cotinine groups.

Smoking Status and Serum Cotinine Levels

Tobacco smoking was reported by ~13.2% of the interviewed women (8% admitted to smoking more than five cigarettes a day). Almost half of the nonsmoking women reported daily exposure to ETS. The most common source of ETS exposure was the smoking by family members (30.3%) and smoking at the workplace (13.1%). The results of cotinine determinations indicated that 20% of the 50 persons presumed to be passive

Table 1 Maternal and Pregnancy Characteristics for the Study Groups Classified According to Serum Cotinine Levels

	Serum Cotinine Levels (ng/mL)			
Variable	**< 2.0 (n = 8)**	**2.0–14 (n = 84)**	**> 14 (n = 22)**	***p***
Maternal characteristics				
Average maternal age, y (SD)	28.0 (4.9)	26.6 (4.9)	24.6 (4.2)	0.146
Average maternal prepregnancy weight ± SD	57.9 ± 11.1	58.8 ± 9.0	59.3 ± 8.9	0.074
Primary education, n (%)	0 (0.0)	10 (12.2)	8 (36.4)	0.932
Unmarried, n (%)	0 (0.0)	18 (21.4)	6 (27.3)	0.27
Pregnancy characteristics				
Average gestational age at the time of examination (wk) ± SD	21.6 ± 3.9	22.6 ± 3.3	23.7 ± 5.1	0.350
Fetal male gender, n (%)	5 (62.5)	44 (52.4)	8(36.4)	0.31
Primiparous, n (%)	2 (25.0)	44 (52.4)	13 (59.1)	0.25
Pregnancy outcomes				
Average birthweight of newborn (g) ± SD	3280 ± 461	3362 ± 425	3165 ± 568	0.068
Average gestational age at delivery (wk) ± SD	39.3 ± 1.6	39.5 ± 1.6	39.1 ± 1.6	0.137
SGA < 10 percentile, n (%)	0 (0)	4 (4.8)	2 (9.1)	0.57

SD, standard deviation; SGA, small for gestational age.

Table 2 Relationship between Serum Cotinine Level and the Mean Crude and Adjusted Values of Fetal Biometry Assessed between 20 and 24 Weeks of Gestation

		Fetal Biometry in 20–24 wk of Gestation					
		Crude Values (Mean ± SD)			Adjusted Values*		
Serum Cotinine Level (ng/mL)	n	BPD	AC	FL	BPD	AC	FL
< 2.0	8	54.9 ± 10.2	165.5 ± 38.9	37.5 ± 38.9	50.2	152.7	34.0
2.0–14	84	57.0 ± 10.1	180.1 ± 36.4	39.6 ± 8.1	49.7	152.9	33.6
> 14	22	59.0 ± 13.1	190.0 ± 49.8	41.6 ± 11.5	48.6	153.2	32.9
p	0.611	0.309	0.481	0.052	0.681	0.133	

SD, standard deviation; BPD, fetal biparietal diameter; AC, abdominal circumference; FL, femur length.
*Adjusted BPD, AC, and FL values were calculated for the mean value of serum cotinine concentration in each group, 20 weeks of gestation, and female fetus gender, using linear regression model.

smokers were actually actively involved in smoking. On other hand, in the group of 49 women reporting to be nonsmokers not exposed to ETS, the cotinine measurements confirmed this status only in six (12.2%) cases, whereas 43 women (87.8%) appeared to be passive smokers.

Ultrasound Fetal Biometric Measurement and Serum Cotinine Levels

No significant differences were found among the three examined groups (classification based on cotinine level) with respect to the mean BPD, AC, and FL values (Table 2). This could be explained by a slightly older gestational age in the group with the highest cotinine levels. However, after controlling for pregnancy duration and fetal gender in the linear model, a significant negative correlation was found between cotinine concentration and fetal BPD. No correlation of this kind was observed for the AC or FL parameters. The values of respective fetal biometric parameters, calculated using the linear regression model, are listed in Table 2.

Qualitative Indices of Umbilical Blood Flow and Serum Cotinine Levels

Serum cotinine level positively correlated with all blood flow indices under study. The highest mean values were noted among women classified as active smokers according to their serum cotinine level, and the lowest mean values were among nonsmokers without ETS exposure, with the values for passive smokers in between (Table 3). However, only the differences in the mean values of S/D and PI reached statistical significance.

A linear regression model was constructed to evaluate the relationship between serum cotinine levels and umbilical blood flow indices (Table 3). In the model we also included the fetal FL and fetal gender as the confounding variables. Our previous studies (the findings not shown) indicated that the longer FL, the lower the value of the umbilical artery S/D ratio. On the other hand, the mean value for this index was higher among female fetuses compared with the male fetuses. Cotinine level was found to be a significant predictor for all of the examined UA blood flow indices. On the basis of the linear regression model, adjusted values of three umbilical blood flow indices were calculated for the value of median cotinine concentration in each cotinine group (Table 3).

S/D Index and Infant Birthweight

To evaluate the prognostic value of S/D index, a linear regression model was developed, with birthweight as the dependant variable, and pregnancy duration, S/D, and infant's gender as the independent variables. An increase

Table 3 Serum Cotinine Level Measured between 20 and 24 Weeks of Pregnancy and the Mean Crude and Adjusted Values of Umbilical Artery Blood Flow Indices

		Umbilical Artery Blood Flow in 20–24 wk of Gestation					
		Crude Values (Mean ± SD)			Adjusted Values*		
Serum Cotinine Level (ng/mL)	n	S/D	RI	PI	S/D	RI	PI
< 2.0	8	2.86 ± 0.28	0.65 ± 0.04	1.07 ± 0.09	2.93	0.66	1.15
2.0–14	84	3.07 ± 0.54	0.67 ± 0.06	1.17 ± 0.17	3.18	0.67	1.20
> 14	22	3.53 ± 0.97	0.68 ± 0.11	1.25 ± 0.22	3.66	0.70	1.30
p		0.006	0.496	0.038	0.003	0.004	0.004

SD, standard deviation; S/D, systolic/diastolic index; RI, resistance index; PI, pulsatility index.
*Adjusted S/D, RI, and PI values were calculated for the mean serum cotinine level in each cotinine group, 20 weeks of gestation, male fetus gender, and femur length = 39.9, using linear regression model.

Table 4 Relationship between Umbilical Artery S/D Ratio Assessed in 20–24 Weeks of Gestation and Infants' Birthweight: Crude Values and Mean Adjusted for Pregnancy Duration

	Birthweight (g)			
	Number of Subjects	Crude Value		Mean Adjusted for Pregnancy
S/D Value	Examined	Mean	SD	Duration
1–2.5	10	3346	465	3371
2.5–3.0	43	3464	456	3484
3.0–3.5	35	3343	362	3438
3.5+	26	2984	462	3128
p		0.001		0.01

S/D, systolic/diastolic index.

in the umbilical artery S/D ratio > 3.0 in 20 to 24 weeks of pregnancy negatively correlated with infant birthweight (Table 4). When the birthweight values obtained for each of the S/D values were adjusted for pregnancy duration, a 243-g difference was found between the lowest and highest S/D group.

DISCUSSION

The findings of this study demonstrated a significant increase in the umbilical artery S/D velocity ratio[Q4] with increasing serum cotinine levels, both for the active and passive smokers. It is noteworthy that as the serum cotinine level increases, all of the qualitative indices of umbilical blood flow increase, which suggests an increasing vascular resistance in the placental circulation both among active and passive smokers.

Newnham et al[25] observed a decreased fetal BPD diameter in the pregnancies of smokers, with a maximal effect at 24 weeks gestation and only among male fetuses. This effect was not associated with an altered head circumference after birth. Our results indicate that the mean fetal BPD measurements are lower, not only in the group of smokers, but also among women exposed to ETS as measured in 20 to 24 weeks gestation.

Findings consistent with the results of our study were reported by Lymeropoulou et al,[21] who showed a significant increase in uteroplacental vascular resistance related to increased cotinine levels. Kimya et al[28] investigated an acute effect of smoking a single, standard 100-mm, filtered cigarette on uterine artery and UA blood velocity waveforms among pregnant women at the mean gestational age of 28 weeks. The results suggest that there was no significant change in the uterine artery and UA blood velocity waveform indices that could be attributed to the acute effect of smoking, but all uterine artery indices and S/D ratio in UA were statistically higher in the smoking women compared with nonsmokers, both before and after smoking. Newnham et al[25] observed no differences in S/D ratios in UA between smokers and nonsmokers, which would indicate that the effects of smoking on the placental vascular resistance were periodic rather than continuous. This is contradictory to the results of our study. Our analysis revealed that either active or passive smoking might have an adverse effect on UA blood flow as early as in midgestation, which implies that the impact of smoking might begin in early pregnancy. According to Morrow et al,[29] the smoking of one cigarette has no significant impact on vascular resistance of the uterine artery (S/D), but is connected with a highly significant increase in S/D ratio in UA as measured between 36 and 41 weeks of gestation. This observation would suggest that active smoking might induce a direct increase in vascular resistance of the placenta on the fetal side.

The results of the present study indicate that exposure to tobacco smoke in pregnancy may induce a direct increase in the vascular resistance of the placenta as early as at midgestation, and this pathomechanism may contribute to the decreased birthweight of the newborns. The analysis also revealed that an increase in the umbilical artery S/D ratio > 3.0 in 20 to 24 weeks gestation significantly correlated with the decreased birthweight.

The negative impact on fetal growth could be explained by several different mechanisms. By changing the morphology and function of the placenta,[30] smoking in pregnancy may lead to fetal hypoxia through placental vasoconstriction. A reduction in uterine blood flow has been demonstrated in animal and human models.[23,24] In the study conducted by Clark and Irion,[23] pregnant ewes received maternal intravenous infusions of nicotine, which produced an increase in fetal blood pressure, a decrease in fetal heart rate, and a decrease in umbilical blood flow, with insignificantly altered S/D ratios. The differences in the placental enzyme activity between active and passive smokers, to some extent, could also explain the adverse effects of relatively low exposure levels observed among passive smokers. According to Remmer,[6] in active smokers, the induction of placental enzymes protects the fetus, whereas the small amount of smoke in passive smokers is unable to induce the enzymes and the smoke remains undetoxified[Q5]. The results obtained by Jauniaux et al[31] indicate that higher cotinine levels could be found in fetal fluids and serum than in maternal serum, and that cotinine accumulated in fetal compartments as early as 7 weeks gestation in both active and passive smokers. As revealed by the findings of Steuerer et al,[32] smoking increases the consumption of vitamin E, hence there is an overproduction of peroxides and a reduction in prostacyclin production, which may lead to a reduced perfusion of the placenta.

The results of this study indicate that intrauterine growth could be negatively affected by tobacco smoke exposure during pregnancy. Doppler examination should

be regarded as an additional diagnostic tool in intrauterine fetal evaluation, and cannot, in itself, be considered a determinant of future fetal growth and development. However, the results of UA Doppler examination in early pregnancy can be used for the assessment of the risk of fetal hypoxia in the populations exposed to specific harmful agents.

CONCLUSIONS

1. The findings of this study suggest that the impaired umbilical blood flow among actively and passively smoking pregnant women begins early in pregnancy and could be one of the mechanisms through which tobacco smoke exposure can affect fetal growth.
2. Prevention measures need to be undertaken to encourage pregnant women to stop smoking and avoid passive exposure to tobacco smoke during pregnancy. Educational activities should be provided to the general population to increase social awareness of the adverse effects, not only of active but also of passive smoking, during pregnancy.

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