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Cotinine concentrations in amniotic fluid and urine of smoking, passive smoking and non-smoking pregnant women at term and in the urine of their neonates on 1st day of life

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Abstract. Cotinine was measured in the amniotic fluid and urine of 31 pregnant women and in the urine of their offspring. Amniotic fluid cotinine was 8 times higher in active and 2.5 times higher in passive smokers than in non-smokers. In general, amniotic fluid cotinine was considerably higher than urinary cotinine both in active and in passive smokers. Estimation of cotinine both in amniotic fluid immediately before delivery and in urine of the newborn on the 1st day of life aids in assessing the degree of prenatal exposure to tobacco smoke.

Key words: Pregnancy – Fetus – Neonate – Smoking and passive smoking – Cotinine concentrations in amniotic fluid and urine

Introduction

Since the recognition of tobacco smoking as a health hazard, concern has been directed toward the consequences for non-smokers exposed to environmental tobacco smoke (ETS). This is a major indoor pollutant to which many children and even fetuses are exposed. Depending on the maternal smoking mode (active or passive) her unborn child becomes a secondary or tertiary smoker [34].

The deleterious influence of smoking by pregnant women on the course of pregnancy and perinatal development of the fetus and newborn [6] includes difficulty in conceiving [13], stress on mother and fetus [20, 25], spontaneous abortion [15], stillbirth [22], preterm birth [31] and low birth weight [31]. Children of smoking mothers have a higher incidence of respiratory disorders [35], increased frequency of asthmatic attacks [10], reduction in the rate of the development of lung function [17], unfavourable mutations [28] or risk of malignancies [26]. Other effects are "sudden infant death" [3], higher neonatal mortality [4] and small but measurable deficiencies in physical growth [27], intellectual and emotional development [16, 24, 30].

Cotinine concentration in body fluids appears to be the best single biochemical short-term marker for chronic exposure to cigarette smoke because of the relatively long half-life of 20–40 h, average urine half-elimination time 68 h (37–160 h) and its relative stability during exposure to smoke [5, 8, 9, 21, 31].

Cotinine concentrations have been measured in amniotic fluid mainly in the 2nd trimester of pregnancy [12, 18, 34, 36], but there is little information about cotinine levels in late pregnancy [1]. This may be important because the original volume and fate of amniotic fluid is different at different stages of pregnancy, as is the vulnerability of the fetus. Also, the simultaneous determination of cotinine levels in pregnant women and their offspring deserves special attention.

This study was undertaken to estimate cotinine concentrations in the amniotic fluid and urine of smoking, passive smoking and non-smoking pregnant women at term and in the urine of their neonates on 1st day of life.

Materials and methods

A group of 31 pregnant women without obstetric or medical complications admitted for delivery were studied together with their neonates, born at 38–42 weeks gestation with an average birth weight of 3351 g.

The subjects were divided into three groups: (1) "non-smokers" – non-exposed to tobacco smoke at home (controls); (2) "passive smokers" – exposed to ETS resulting from other persons' tobacco smoke; and (3) "active smokers" – smoking an average of 15 cigarettes/day. The latter two groups inhaled tobacco smoke during the last days and even the last 24 h before delivery.

Urinary cotinine was analysed by a slightly modified [14] direct colourimetric method (DBA) according to Barlow et al. [2]. For amniotic fluid the method was adapted by additional preliminary and final centrifugation of the sample at room temperature (825 $\times g$ for 10 min). A blank was run parallel to the assay. Individual

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Abbreviations: DBA = direct barbituric acid method; ETS = environmental tobacco smoke

Table 1. Cotinine and creatinine concentrations in the amniotic fluid and urine of smoking pregnant women and their neonates

Pregnant women at term	Statistical symbols	Age (years)	Amniotic fluid			Maternal urine			Neonatal urine			
			Cotinine (μmol/l)	Creatinine (mmol/l)	Cotinine/ creatinine ratio (μmol/mmol)	Cotinine (μmol/l)	Creatinine (mmol/l)	Cotinine/ creatinine ratio (μmol/mmol)	Age (week gestation)	Cotinine (μmol/l)	Creatinine (mmol/l)	Cotinine/ creatinine ratio (μmol/mmol)
I	*n*	8	8	8	8	8	8	8	8	8	8	8
Non-exposed	$\bar{x}$	26	15	0.36	42	14	13.3	1.0	40	13	3.4	3.8
("non-smokers")	SD	3	3	0.06	14	4	5.3	0.7	2	3	0.6	0.5
	Median	26	14	0.35	41	14	13.3	0.9	41	13	3.3	3.7
	Range	19–31	3–20	0.23–0.46	24–70	8–20	7.2–23.7	0.3–2.8	36–42	8–16	2.6–4.2	3.1–4.7
II	*n*	14	14	14	14	14	14	14	14	14	14	14
Exposed at home	$\bar{x}$	25	37	0.36	103	17	11.9	1.4	39	18	3.2	5.6
("passive smokers")	SD	3	9	0.07	31	5	3.1	0.6	1	4	0.5	1.4
	Median	25	37	0.35	105	18	11.7	1.2	40	17	3.2	5.5
	Range	19–31	25–58	0.23–0.49	65–154	9–26	8.0–18.6	0.6–2.8	38–42	13–25	2.5–4.3	3.2–9.0
III	*n*	9	10[a]	10[a]	10[a]	9	9	9	9	9	9	9
Active smokers	$\bar{x}$	24	111	0.35	317	53	10.7	4.9	39	44	3.2	13.8
	SD	6	64	0.08	176	36	3.6	3.6	2	18	0.7	7.5
	Median	25	88	0.33	270	39	9.3	5.0	40	40	2.8	12.9
	Range	16–35	51–274	0.22–0.46	111–668	20–127	7.4–19.6	1.1–13.9	35–41	22–90	2.4–4.3	8.7–34.2
	I/II		<0.001	>0.05	<0.001	>0.05	>0.05	>0.05		<0.01	>0.05	<0.001
P	I/III		<0.001	>0.05	<0.001	<0.01	>0.05	<0.001		<0.001	>0.05	<0.002
	II/III		<0.002	>0.05	<0.001	<0.01	>0.05	<0.01		<0.001	>0.05	<0.01

[a] The values of alive-born and still-born twins (in separate amniotic sacks) are included
SD = Standard deviation

blank values (the interference arising from the natural colour of body fluids) did not exceed 0.05 absorbance units at 506 nm.

The assay was standardised using aqueous solutions of 0–250 μmol/l cotinine (Sigma, C9399) and the results were expressed as "μmol/l cotinine equivalents". The assay was linear up to 250 μmol/l.

The intra- and interassay variations for both low and high amniotic fluid cotinine concentrations were 4.2% ($n = 10$) and 6.7% ($n = 10$ days), respectively. Recovery of 5 μmol cotinine added to amniotic fluid samples with a cotinine concentrations of 28 and 81 μmol/l was close to 100% ($n = 8$). The sensitivity of the assay was established using standard solutions of decreasing concentration. The lower limit of detection of cotinine was 0.65 μmol/l.

Creatinine was determined according to Siedel [33] using a commercially available kit (Boehringer-Mannheim, Mannheim, FRG).

Amniotic fluid samples were collected at vaginal delivery after spontaneous or surgical rupture of the membranes. Blood-stained samples were discarded. Urine specimens from neonates were obtained with the first 8 h of life. Analyses were performed on the same day.

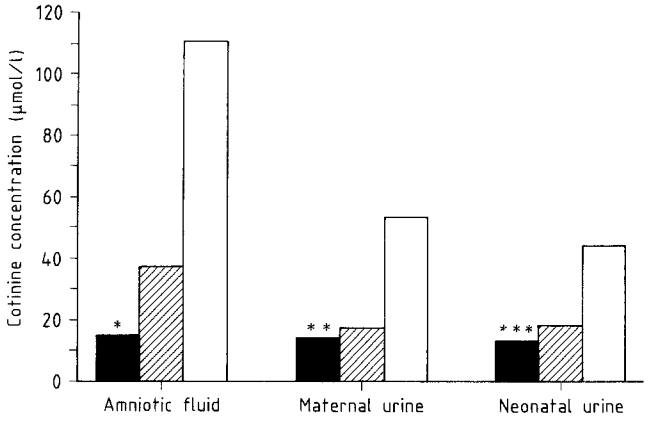

Fig. 1. Cotinine levels in amniotic fluid and urine of pregnant women at term (■, non-exposed "non-smokers"; ▨, passive smokers; □, active smokers) and in urine of their neonates during the first 8 h of life. * $P < 0.001$; ** $P > 0.05$; *** $P < 0.01$

Results

Mean cotinine concentrations and cotinine/creatinine ratios in amniotic fluid and urine of pregnant women at term and their neonates are presented in Table 1.

Cotinine concentrations were low in the amniotic fluid and urine of non-smokers, as well as in the urine of their neonates.

Passive smokers had urinary cotinine values close to those of non-smokers ($P > 0.05$), however, their amniotic fluid cotinine levels were twice those found in non-smokers ($P < 0.001$). Neonates born to passive smokers had higher concentrations of urinary cotinine than those of non-smokers ($P < 0.01$) (Fig. 1).

An unexpected finding was the considerably higher amniotic fluid cotinine levels compared to urinary cotinine in both active and passive smokers.

Discussion

Though nicotine, pyridil acetic acid and nicotine N-oxide may interfere with the DBA determination of cotinine, the reliability of the DBA method used in this study is undisputed [2].

Accordingly, the present results are similar to those for cotinine in urine [2, 8, 9, 19, 21, 23, 29] and amniotic fluid estimated by radioimmunoassay [36], gas chromatography-mass spectrometry [1, 19] or gas-liquid chromatography [34].

The results indicate that low (3–20 μmol/l) concentrations of cotinine are found in non-smokers. This could be due to inaccuracy in self-reported smoking status or to the occasional exposure to tobacco smoke in public places. Approximately one-third (about 2.5 million) of the adult population in Bulgaria smokes and cigarette smoke exposure is often almost unavoidable.

Urinary cotinine concentrations in pregnant women and their offspring were similar to those of non-smokers and passive smokers (Fig. 1). On the other hand, amniotic fluid cotinine in passive smokers was more than twice, and that in moderately severy active smokers about 8 times as high as in controls.

The higher level of cotinine in amniotic fluid compared to the urine of active and passive smokers is unexplained. It is not clear whether cotinine in amniotic fluid is a metabolite produced by the fetus itself from passively acquired nicotine, whether it is simply maternally derived cotinine transferred across the placenta, or both [34]. Nicotine is converted to cotinine in the liver [11].

Our study confirms the reliability of the cotinine/creatinine ratio in body fluids as an index of the degree of tobacco exposure.

Cotinine itself is not the substance of major concern in respect to health. It is an indirect marker of the exposure to a large variety of other, more damaging tobacco smoke components derived from the "mainstream" in smoking women and from the "sidestream" smoke in passive smokers. Mainstream and sidestream smoke are quite different in composition in that sidestream smoke contains more potentially carcinogenic substances than mainstream smoke [7].

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