Drug and Alcohol Dependence 134 (2014) 275–279

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

High cotinine levels are persistent during the first days of life in newborn second hand smokers

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ARTICLE INFO

Article history:
Received 21 June 2013
Received in revised form
17 September 2013
Accepted 20 October 2013
Available online 31 October 2013

Keywords:
Maternal smoking
Umbilical cord blood cotinine
Birth weight
Second hand smoke

ABSTRACT

Background: Despite the adverse effects of maternal smoking during pregnancy on the newborn's health are well-known, in the pediatric population, a high prevalence exists that is very much affected by second hand smoke (SHS). This study aims to investigate the impact of maternal smoking habits during pregnancy on cotinine levels in newborns during the first days of life. The high association between cotinine concentration in maternal and umbilical cord blood (UCB) has been previously reported, but the levels of blood cotinine that remain in infants born to smokers is unknown.
Methods: Cotinine concentration was measured in UCB, in maternal and newborn peripheral blood. Data from UCB sample dyads of ninety mothers and from seventy-one newborns were analyzed.
Results: Cotinine levels were significantly different among non-smokers (9.9 ± 5.9 ng/ml), moderate (67.3 ± 7.4 ng/ml), and heavy smokers (137.7 ± 19.5 ng/ml) ($p < 0.0001$). Significant correlations were found between maternal and UCB cotinine ($r = 0.748$; $p < 0.001$), and between UCB and newborn plasma cotinine at 48 h after birth ($r = 0.541$; $p < 0.001$). The smokers exposed their infants to cotinine with a median of 31.7 ± 8.6 ng/ml (moderate) or 59.1 ± 13.3 ng/ml (heavy smokers) until at least, 48 h after birth. Reduced birth weight and length were significantly related with UCB cotinine levels.
Conclusions: A positive association between UCB and plasmatic cotinine in newborns was found. The high cotinine levels detected in newborns from smoker mothers indicates that their infants are subjected to elevated SHS from birth. These results can help to reinforce the awareness of the adverse effects of smoking during pregnancy.

1. Introduction

Events that occur during critical periods, such as gestation, can influence future health and disease. Maternal exposure to one of the most common pollutants, cigarette smoke, plays an important role and is considered a risk factor for perinatal mortality, low birth weight, and neurological abnormalities (Bearer et al., 1997; Mamun et al., 2012; Bergen, 2006). Despite the fact that smoking during pregnancy has adverse effects, the prevalence of young female smokers remains high, between 30 and 36% in Spain (Jiménez Ruiz, 2006; Pichini et al., 2000). Even though Spain implemented legislation to reduce second hand smoke (SHS) in 2005, 16% of pregnant women are smokers (Puig et al., 2012). Consequently, the percentage of newborns potentially affected by maternal smoking during pregnancy is elevated in our geographical area (in the region of Valencia, Spain) with important public health implications (Chiolero et al., 2005; Bakker and Jaddoe, 2011; Jaakkola and Gissler, 2004; Ino et al., 2011; Raum et al., 2011; Gospe et al., 1996; Law et al., 2003).

Intrauterine tobacco exposure is usually evaluated by a questionnaire administered to pregnant women or by biomarker detection. The self-reported questionnaire has been extensively used to evaluate smoking status but is a method that is frequently unreliable. Different studies have shown that self-reports tend to underestimate these assessments due to social stigma (Gorber et al., 2009). The use of biomarkers constitutes the most objective method of ascertaining tobacco exposure. Cotinine is a by-product of nicotine metabolism and is considered the most suitable biological marker for determining smoking habits at the end of pregnancy (Llaquet et al., 2010). Cotinine crosses the placenta and its

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http://dx.doi.org/10.1016/j.drugalcdep.2013.10.017

Table 1
General characteristics of mother and newborns grouped according to cotinine umbilical cord blood levels.

	Slightly exposed ($n=36$) Cotinine < 14 ng/ml	Moderately exposed ($n=37$) Cotinine 14–100 ng/ml	Highly exposed ($n=17$) Cotinine > 100 ng/ml	p-value
Maternal characteristics				
Maternal age (years)	28.7 ± 0.9	30.2 ± 1.0	31.9 ± 119.0	0.139
Cotinine levels (peripheral blood) (ng/ml)	9.9 ± 5.9	67.3 ± 7.4	137.7 ± 19.5	$p<0.0001$
Newborns characteristics				
Cotinine levels (UCB) (ng/ml)	1.4 ± 0.5	49.9 ± 3.9	173.1 ± 11.2	$p<0.0001$
Gestational age at delivery (wk)	38.9 ± 0.2	39.1 ± 0.2	39.4 ± 0.3	0.409
Sex (male/female)	22/14	16/21	10/7	0.324
Breastfeeding (%)	58.3	62.2	76.5	0.536
Birth weight (g)	3415.1 ± 100	3100.1 ± 75	3027.1 ± 80	$p<0.05$
Height (cm)	49.4 ± 0.4	48.7 ± 0.3	47.6 ± 0.5	$p<0.05$
Systolic blood pressure (mm Hg)	74.7 ± 1.8	73.2 ± 1.8	75.2 ± 2.8	0.791
Diastolic blood pressure (mmHg)	46.3 ± 1.6	44.8 ± 1.6	46.8 ± 2.4	0.761
Heart rate (bpm)	124.94 ± 2.2	119.8 ± 2.3	127.0 ± 3.2	0.150

The values are mean ± SE; p-value = statistical significance of the differences among groups.

concentration in cord blood can be indicative of tobacco consumption (Luck et al., 1985; Berlin et al., 2010). The objective of the present study was to investigate cotinine levels in umbilical cord blood (UCB) and their persistency after birth in newborn second hand smokers. The impact of cotinine levels on anthropometric parameters and blood pressure values was also assessed.

2. Methods

2.1. Design

The study was performed at the Hospital General Universitario in Valencia, Spain between September, 2010 and June, 2011. The eligibility criteria for pregnant women were prenatal care without complications during pregnancy (for example gestational diabetes or hypertension among others). All the mothers were healthy and had no cardiovascular risk factors, except those who were active smokers. Eligibility by smoking status was determined through maternal self-report and blood cotinine levels. Prior to participation, women read and signed an informed consent.

UCB samples were obtained from the clamped umbilical cord immediately after delivery and 48 h after birth. Samples from the mothers were drawn 24 h after delivery. The samples and collected data were stored according to the directives dictated by the law of Biomedical Investigation of 2007 (Law 14/2007). The study was approved by the hospital's review board and was carried out in accordance with the Declaration of Helsinki. A birth cohort of 90 mother–newborn dyad subjects was included in the study.

2.2. Sample collection

UCB samples were obtained in newborns at term (gestational age ≥37 weeks) (Ballard et al., 1979) after uncomplicated pregnancy and in the absence of perinatal illness. UCB, maternal and newborn peripheral venous blood were collected in EDTA-tubes, centrifuged to yield plasma, stored at −80 °C and thawed before use.

2.3. Cotinine analysis

Tobacco exposure was measured using a bioassay for cotinine. Concentrations of cotinine were determined in plasma samples by ELISA according to the manufacturer's recommendations (Salimetrics, SPK 1-2002-5). The minimal concentration that can be distinguished is 0.05 ng/ml.

2.4. Data analysis

Experimental values are expressed as mean ± SE. Analysis of variance (ANOVA) was used to compare differences between groups. A significant difference was considered present if $p<0.05$. The relationship between cotinine levels in peripheral maternal blood, venous umbilical cord blood, and plasma cotinine in newborns was examined by Pearson's correlation coefficient.

The correlations between cotinine in cord blood and birth weight and length were examined by partial correlation and adjusted by sex and gestational age. Statistical analyses were performed using SPSS 15.0 (SPSS Inc, Chicago, Illinois, USA) and GraphPad Statmate 5.0 (GraphPad Software, La Jolla, California, USA) software graphs.

3. Results

A total of 90 mother–infant dyads were included in the study. A combined assessment of biomarker and self-reported measures were used to confirm smoking status and prenatal exposure. Of the 90 mothers, 54 were active smokers (60%) and 36 were non smokers (40%, cotinine levels <14 ng/ml). Of the 54 smokers, 37 (41.1%) were moderate smokers (cotinine levels between 14 and 100 ng/ml) and 17 (18.9%) were heavy smokers (cotinine levels >100 ng/ml).

To differentiate between low and high SHS exposure in newborns, a cut-off of 14 ng/ml was used, according to the classification values described by Pichini et al. (2000). Three groups of newborns were considered; low (0–14 ng/ml), moderate (14–100 ng/ml) and high exposure (>100 ng/ml) (see Table 1). Anthropometrical characteristics and smoking status for each group are summarized in Table 1. On average, infants were born at 38.9 ± 1.6 weeks of gestation and weighed 3228.5 ± 609.5 g. Newborns with moderate and high exposure had a lower birth weight (3100.1 ± 75 g and 3027.1 ± 80 g respectively) than newborns with low exposure (3415.1 ± 100 g; $p<0.05$).

No significant differences were observed in maternal age, gestational age, gender, feeding (breast or formula) systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate among the 3 groups of newborns. However, we found a significantly shorter height in infants whose mothers were smokers (48.7 ± 0.3 cm in moderate and 47.6 ± 0.5 cm in heavy smokers) versus non-smokers (49.4 ± 0.4 cm; $p<0.05$) (see Table 1). The Pearson correlation between APGAR score and cotinine levels shows an inverse and significant correlation with cotinine at 48 h ($r=-0.243$; p-value = 0.042). No correlation was found between APGAR values and maternal or umbilical cord blood cotinine.