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Nicotine Analysis in Neonates' Hair for Measuring Gestational Exposure to Tobacco

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ABSTRACT: Hair samples were collected at time of delivery from 40 neonates whose mothers were known to be smokers during the prenatal period. Hair was decontaminated in dichloromethane, homogenized in NaOH, and nicotine was extracted in diethyl ether. After separation on a BP-5 capillary column, nicotine was identified and quantified by GC/MS using selected ion monitoring. In all cases, nicotine was found in the neonatal hair and in the hair of the corresponding mother. The ranges of nicotine levels were 0.15 to 11.80 ng/mg, and 0.37 to 63.50 ng/mg, for the neonates, and their mothers, respectively. It was possible to establish a significant correlation between both concentrations, and the correlation coefficient was 0.83. These findings suggest the possibility of monitoring the transfer of maternal nicotine through the placenta by measuring nicotine concentration in neonatal hair.

KEYWORDS: toxicology, tobacco, nicotine, hair, neonatal hair, gestational exposure

Maternal smoking is a health hazard for the fetus. Babies born to women who smoke during pregnancy weigh about 200 g less than babies born to non smoking women, and they also have an increased risk of spontaneous abortion, and fetal death. Neonatal death increases as does the frequency of abruptio placentae, and placenta previa. Sudden infant death syndrome is more common among infants of smoking mothers. Children born to smoking mothers develop more slowly physically and mentally through the teen years. The relation between maternal smoking and low birth weight is nicotine-dose dependent and low birth weight is due to the direct retardation of fetal growth, and is marked by decreases in length and in the circumferences of the head, chest and shoulders. Smoking increases the level of carboxyhemoglobin in both maternal and fetal blood, with subsequent reductions in oxygen binding capacity, resulting in fetal hypoxia [*1–9*].

Components of cigarette smoke are readily transported over the placental membrane and bioactivation of procarcinogens to mutagens in human fetal and placental tissues has been demonstrated [*10–12*].

Because of these immediate and long-term problems, newborns born to women exposed to nicotine during pregnancy should be identified soon after birth so that appropriate intervention and follow-up can be done.

Failure to identify tobacco exposed users is extensive owing to the limitations of the three methods currently used to verify drug use. Maternal self-reported drug history, the

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first method, has been shown to be unreliable. Many women who deny use during pregnancy exhibit drug metabolites in their urine. Moreover, systematic urinalysis, the second method, is hampered by the short elimination half-life of the drugs. This test is not suitable for validation of survey data, since the quantification of drugs in urine only reflects exposure during the preceding 1 to 3 days and does not necessarily indicate the frequency, in subjects who might deliberately abstain for several days before biomedical screenings. Moreover, comparison of drug concentrations in umbilical cord blood, neonatal blood or amniotic fluid measured at delivery and in maternal blood cannot provide information on the duration and degree of fetal exposure. The same disadvantages are noted with the analysis of meconium, which is only a qualitative test at the moment of delivery [*13,14*]. Recently, gestational exposures to cocaine [*15*] haloperidol [*16*] and morphine [*17*] were revealed by hair analysis of neonates. Hair analysis may remedy the disadvantages of currently used methods with a wide window of detection ranging from weeks to months and may provide information concerning the severity and pattern of an individual's drug use, when a maternal drug history is not available or in doubt. In this study, we measured nicotine in hair collected from neonates and their smoking mothers.

Material and Methods

Hair samples and drug histories were obtained from 40 pregnant women with self-reported use of tobacco and from their babies admitted at the "Service de Pédiatrie" at the Hautepierre Hospital, Strasbourg, France. Hair samples were collected at the time of delivery or 1 to 5 days after delivery, after the nature of the study was explained and the mother had given her verbal informed consent.

The strands of hair were simultaneously sampled from the mother (about 50 mg) and the neonates (7 to 37 mg) by cutting with small scissors close to the scalp. Until analysis, the hair was placed into a separate plastic tube and stored at ambient temperature.

The hair was decontaminated by washing in 5 mL dichloromethane for 15 min at 37°C. The washed hair was homogenized in a capped tube in 1 mL of 1 N NaOH for 10 min at 100°C. After cooling, nicotine was extracted using 5 mL diethyl ether in the presence of 20 μL of ketamine (1 mg/L) as an internal standard. After agitation and centrifugation, the organic phase was removed. 20 μL octanol were then added to ensure nonvolatility of nicotine. After rapid evaporation of the diethyl ether, the residue was dissolved in 15 μL dichloromethane and 2 μL of the solution was injected into a 12 m by 0.22 mm inside-diameter BP-5 capillary column. The flow rate of the carrier gas (helium N55) through the column was 3.2 mL/min. The column oven temperature was programmed from an initial temperature of 60°C (held for 0.9 min) to 280°C at 30°C/min and held for the final 1 min. Splitless injection with a split valve off-time of 0.9 min was used.

The gas chromatographic system consisted of a Perkin Elmer (8500) chromatograph with an Ion Trap Detector (ITD). The ITD was operated in the electron impact mode at 70 eV with an ion source temperature of 210 to 220°C. The electron multiplier voltage of the detector was set in the range 100 to 200 V above auto tune voltage. The instrument was autotuned daily with $C_{12}F_{27}N$. For detection and quantification, selected single ion monitoring was used as follows: nicotine, m/z 84 and ketamine, m/z 180. Calibration curves were prepared using the results of analyses homogenates of guinea pig's hair spiked with nicotine.

The assay had a >80 % extraction efficiency and the limit of detection was approximately 0.01 ng/mg of hair using a sample of 10 mg of hair [*18*].

Results and Discussion

Very few reports dealing with the analysis of nicotine in human adult hair seem to be published, most involving few cases [*18–21*]. It was demonstrated that only the nicotine

concentration, not the cotinine concentration, its major metabolite, is the basis for differentiating smokers from non smokers, all the investigations were carried out in evaluating the parent drug [*18*].

Hair from all 40 neonates with a maternal history of tobacco use was positive. Nicotine concentrations ranged from 0.15 to 11.80 ng/mg of hair, most of the levels measured in the range 0.15 to 4.00 ng/mg of hair. In the corresponding mothers, the nicotine concentrations ranged from 0.37 to 63.50 ng/mg of hair. By comparison with a population of adult smokers, some concentrations measured in hair of neonates are particularly elevated, clearly indicating dramatic consumption by the corresponding mother. Passive adult smokers exhibit nicotine concentrations in the range of 0.84 to 1.82 ng/mg of hair [*18*]. Nevertheless, one can also suppose that the difference between the drug concentrations found in the hair of neonates and adults is a result of the different growth and length of hair. Because it is likely that maternal nicotine is secreted into amniotic fluid, the decontamination procedure is essential to remove any drug that may have been adsorbed into hair. It is known that drug passes from the circulating fluids into hair and remains firmly bound there, but nothing is known about incorporation from a medium, that is, the amniotic fluid, where hair is exposed for a long time.

When available, urines of the neonates were collected and then analyzed by fluorescence polarization immunoassay on an Abbott TDx for cotinine, according to the manufacturer's recommendations. Concentrations ranged from non detectable to 83 μg/L most (60%) were negative. This demonstrates that this GC/MS hair analysis technique is better able to detect previous tobacco use than is the above urinalysis immunoassay.

It was possible to establish a significant correlation ($P < 0.001$) between the nicotine concentrations in the hair of the neonates and of their corresponding mothers (Fig. 1). The mathematical expression of the regression is: Y (neonate) = 0.137 × (mother) +

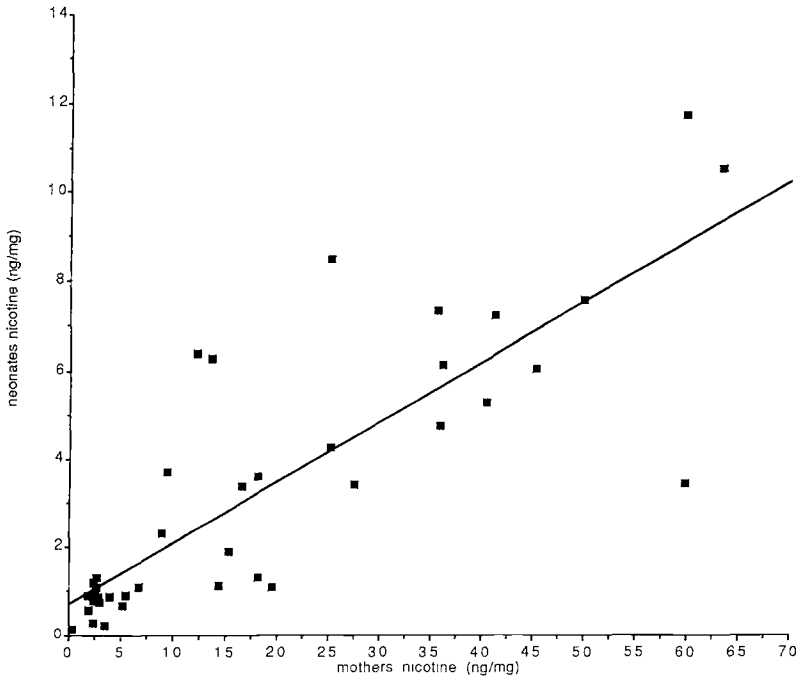

FIG. 1—*Correlation between the nicotine concentration in the hair of neonates and their corresponding mothers.*

0.737, with a correlation coefficient of 0.83. This correlation clearly indicates a dose dependent transfer of maternal nicotine to her baby. The clinical observations of the neonates, based on the incidence of the nicotine measured should be evaluated in further studies.

Conclusion

This study shows that maternal nicotine is transferred to the fetus through the placenta and retained in fetal hair, suggesting that drug analysis of hair of neonates can provide information on fetal exposure to maternal drugs, particularly on the duration of fetal exposure.

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