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Using nicotine in scalp hair to assess maternal passive exposure to tobacco smoke☆

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ABSTRACT

Quantifying population exposure level to tobacco smoke is important for investigating its adverse effects on human health. We aimed to investigate the feasibility and application of using population hair concentrations of nicotine and cotinine to indicate their exposure level to tobacco smoke among pregnant women. Our study recruited 256 mothers who delivered healthy babies and collected their hair samples from scalp, of which 172 mothers were self-reported non-passive smokers and the other 84 mothers were self-reported passive smokers. We analyzed nicotine and cotinine concentrations of the hair section grown during the early pregnancy. The linear relationship between cotinine and nicotine was developed and validated by internal cross-validation method. Our results revealed that self-reported passive smokers had higher concentrations of nicotine [2.08 (1.00–4.46) ng/mg hair, i.e. median value (inter-quartile range)] and cotinine [0.063 (0.041–0.148) ng/mg hair] than non-passive smokers [1.35 (0.58–2.59) ng/mg hair of nicotine and 0.049 (0.022–0.087) ng/mg hair of cotinine, respectively]. There existed a linear regression model between hair cotinine and nicotine concentrations, i.e. [cotinine] = 0.024 × [nicotine]+0.0184 (R^2 = 0.756) for this population. The internal cross-validation squared correlation coefficient slightly increased from 0.689 to 0.734 with the training subjects varying from 20% to 90%, suggesting that this regression model had high robustness and predictive accuracy. It was concluded that nicotine in maternal hair can evaluate the hair cotinine level and reflect maternal passive exposure level to ambient tobacco smoke with high sensitivity.

1. Introduction

Tobacco smoking has been considered as an important risk for adverse maternal and fetal outcomes, such as reduced fertility, low birthweight, and congenital anomalies (Murin et al., 2011; Salmasi et al., 2010). Various toxic pollutants are produced during smoking including carbon monoxide, nicotine, hydrogen cyanide, and polycyclic aromatic hydrocarbons (Eliopoulos et al., 1994). Among them, nicotine was considered as a representative constituent of tobacco smoke, which was used as an indicator of its exposure level of passive or active smokers in environmental health studies (Avila-Tang et al., 2013; Benowitz and Jacob, 1984; Jarvis et al., 1987). Up to 80% of nicotine was metabolized into cotinine and its derivatives after inhaled into human body, so cotinine was the major proximate metabolite of nicotine (Benowitz, 1996). Hence, concentrations of nicotine and cotinine in the biological matrices like serum, hair, saliva, and urine were used to indicate the exposure level of tobacco smoke (Al-Delaimy, 2002; Al-Delaimy et al., 2002).

Compared with other matrices, hair has three important advantages: (1) reflecting long-term accumulated systemic exposure; (2) indicating the exposure level of a specific time window by using the corresponding segments of hair samples by assuming a consistent hair growth rate; (3) non-invasive collection. Therefore,

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hair analysis of nicotine and cotinine has been widely applied in previous studies (Al-Delaimy, 2002; Al-Delaimy et al., 2002; Ashford et al., 2010; Brajenovic et al., 2013; Yoo et al., 2010). In human hair, concentration of cotinine was about one order of magnitude lower than that of nicotine (Eliopoulos et al., 1994; Klein et al., 2004; Seong et al., 2008); Moreover, hair cotinine in passive smokers was about one order of magnitude lower than that of active smokers. It resulted in great challenge to analyze hair cotinine with high accuracy and precision, especially when the hair quantity of specific segments or from little children was insufficient. We observed that some studies only measured nicotine concentration of hair rather than cotinine although mass spectrometer with high sensitivity was utilized (Brajenovic et al., 2013; Jaakkola et al., 2001; Loy and Jan Mohamed, 2014; Yoo et al., 2010). The disadvantage of nicotine analysis is that nicotine in gaseous or particulate phase suspending in ambient air may adsorb on the hair surface. Inefficient washing of adsorbed nicotine during hair treatment may result in a false positive result (Pichini et al., 1997). Up to now, the correlation between nicotine and cotinine in human hair in large population has not been well addressed.

China has the largest number of tobacco smokers in the world with ~300 million citizens of active smoking and ~540 million citizens suffering from passive smoking ("China Report on the Health Hazards of Smoking" by Ministry of Health of the P.R. China, 2012). An estimated 28.1% of adults in China were current smokers (52.9% for the male and 2.4% for the female) (Li et al., 2011), while the prevalence of passive smoking for women was pretty high, i.e. 71.6% (Xiao et al., 2010). Although this prevalence would decrease significantly during pregnancy, 41.9% of them were still exposed to almost the same amount of tobacco smoke with that before pregnancy (Fu et al., 2008). For Chinese women, exposure to environmental tobacco smoke was related to elevated risks of all-cause mortality and mortality due to lung cancer and cardiovascular disease (Wen et al., 2006). Likewise, maternal passive smoking can increase the concentration of cotinine in newborn infants (Almeida et al., 2011; Eliopoulos et al., 1994). Up to now, fundamental data about population exposure to nicotine from tobacco smoking were limited in China. Hence, an efficiency method to evaluate the level of passive smoking of women in China becomes pretty urgent. We therefore proposed the hypothesis that nicotine in maternal hair can indicate their internal cotinine level in maternal body, and both of them can present their passive smoking status. The aims of this study were to investigate the feasibility and application of using population hair concentrations of nicotine and cotinine to indicate the exposure level of tobacco smoke among pregnant women.

2. Materials and methods

2.1. Hair sampling

Hair samples of 256 mothers, recruited in four counties (i.e. Taigu, Pingding, Xiyang, and Zezhou) and Taiyuan city in Shanxi Province, and five counties (i.e. Mancheng, Laoting, Fengrun, Yuanshi, and Xianghe) and Shijiazhuang city in Hebei Province from 2003 to 2007, were collected. All mothers delivered healthy babies without diagnosed birth defects. The concerned questions in the questionnaire were maternal age, ethnicity ("Han" or "others"), occupation ("non-chemical industry worker" or "chemical industry worker"), education ("primary or lower", "junior high", or "high school or above"), gravidity, periconceptional folate supplementation, fever or cold during the periconceptional period, alcohol drinking, and passive smoking status. Definition of passive smoking is staying in the tobacco smoking environment for at least 30 min and at least 1 time per week. Women with active smoking history were excluded. We collected information using face-to-face interviews conducted by trained local health workers. Hair samples from all the mothers were collected using stainless steel scissors, and were cut from the back of the head, as near as possible to the scalp. We sealed the hair samples separately in labeled polyethylene zip-lock bags and stored them at room temperature away from light, which were not opened prior to analysis in the laboratory. The study protocol was approved by the institutional review board of Peking University, and signed consent was obtained from all subjects.

2.2. Analysis of nicotine and cotinine

Raw human hair sample (~25 mg) was cut into segments with the length (3–5 mm) and transferred into a washed 2-mL glass vial for each sample. The overall analysis procedure of nicotine and cotinine was described in our previous study (Li et al., 2016). Briefly, hair samples and blank vials were washed by 1 mL Triton X-100 (vortex for 5 min), 1 time; 1 mL deionized water (vortex for 5 min), 3 times; and further washed using a 1-mL mixture of *n*-hexane (Ultra Resi-Analyzed®, Merck KGaA) and dichloromethane (J.T. Baker®, USA) (3/2, v/v) (noted as "HD32") (vortex for 5 min), 2 times. To confirm the performance of washing procedure, we analyzed the nicotine and cotinine concentrations in washed solutions by using a mixture of hair sample cut from passive smokers. After washing, 0.5 mL tetramethylammonium hydroxide (25% m/v, in H_2O) (TMAH) solution was added into the vial and hair sample was digested in the ultrasonic cleaner (KQ-500 B, Kunshan, China) for 1 h. Then, 0.5 mL HD32 was added to the digestion mixture and extract organic compounds by vortex mixing (1000 rpm, 5 min). The extraction procedure was repeated for three times and the organic phase for each sample was pooled in a vial (~1.5 mL). Add internal standard (i.e. diphenylamine) to the extraction solution for quantifying nicotine and cotinine. Filtrate the extraction solution using Teflon® filter (0.22 μm) to remove the particulate impurities and concentrate the solution to approximately 100 μL via evaporation under nitrogen. The concentrations of nicotine and cotinine were determined using a gas chromatograph (Agilent 7890 B, USA) coupled with a mass spectrometer (MS, Agilent 5977 A) equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The detailed quantification parameters were provided in the section "Quantification Parameters of Nicotine and Cotinine" in Supplementary Materials. The detection limits of nicotine and cotinine were 2 ng/mL and 5 ng/mL, respectively. The detection rates of nicotine and cotinine were 100% and 98%, respectively. Procedure blanks in triplicate were included in each batch of experiments for quality control. Quantitative analysis was conducted in the Central Laboratory of Biological Elements in the Peking University Health Science Center, and the protocol was qualified by the China Metrology Accreditation (CMA) system.

2.3. Data analysis

The concentrations of nicotine and cotinine in hair samples were calculated by subtracting the corresponding mean concentration of the blank controls in each batch. Concentrations below the limit of detection were assigned as zero. Kolmogorov-Smirnov method was used to test normal distribution. The median value with the interquartile range (IQR) was used to describe the skewed distributions. Concentration differences between non-passive smokers and passive smokers were evaluated using the Mann-Whitney *U* test. Maternal characteristics of the passive smokers and non-passive smokers were compared using a Chi-square test. Maternal hair concentrations of nicotine and cotinine were converted into normal distribution by lognormal transformation. The correlation between their logarithm-transformed concentrations