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Slower nicotine metabolism among postmenopausal Polish smokers

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ABSTRACT

Background: A non-invasive phenotypic indicator of the rate of nicotine metabolism is nicotine metabolite ratio (NMR) defined as a ratio of two major metabolites of nicotine – *trans*-3′-hydroxycotinine/cotinine. The rate of nicotine metabolism has important clinical implications for the likelihood of successful quitting with nicotine replacement therapy (NRT). We conducted a study to measure NMR among Polish smokers.

Methods: In a cross-sectional study of 180 daily cigarette smokers (42% men; average age 34.6 ± 13.0), we collected spot urine samples and measured *trans*-3′-hydroxycotinine (3-HC) and cotinine levels with LC–MS/MS method. We calculated NMR (molar ratio) and analyzed variations in NMR among groups of smokers.

Results: In the whole study group, an average NMR was 4.8 (IQR 3.4–7.3). The group of women below 51 years had significantly greater NMR compared to the rest of the population (6.4; IQR 4.1–8.8 *vs.* 4.3; IQR 2.8–6.4). No differences were found among group ages of male smokers.

Conclusions: This is a first study to describe variations in nicotine metabolism among Polish smokers. Our findings indicate that young women metabolize nicotine faster than the rest of population. This finding is consistent with the known effects of estrogen to induce CYP2A6 activity. Young women may require higher doses of NRT or non-nicotine medications for most effective smoking cessation treatment.

Introduction

Nicotine metabolism in humans

Nicotine is the primary addictive compound in tobacco. The extent and variation in nicotine metabolism is important because of the key role nicotine play in producing tobacco dependence and regulating smoking behavior. The liver cytochrome P450 2A6 (CYP2A6) is a key enzyme involved in the nicotine metabolism. In humans, nicotine is converted *via* C-oxidation into cotinine, which is in turn converted by the same enzyme to trans-3′-hydroxycotinine (3-HC) (Fig. 1). 3-HC is excreted in urine conjugated with glucuronic acid (7–9% of absorbed nicotine) and in free form (33–40%). Cotinine is also excreted as free form (10–15% of absorbed nicotine) and cotinine glucuronides (12–17% of absorbed nicotine) [1].

The speed of nicotine metabolism in the body is correlated with a likelihood of successful smoking cessation when supported with nicotine replacement therapy (NRT) [2]. It is known that *CYP2A6* genotype affects plasma levels of nicotine obtained from NRT [3]. This may explain, at least in part, the differential effectiveness of the therapy between patients with slow and fast rate of nicotine

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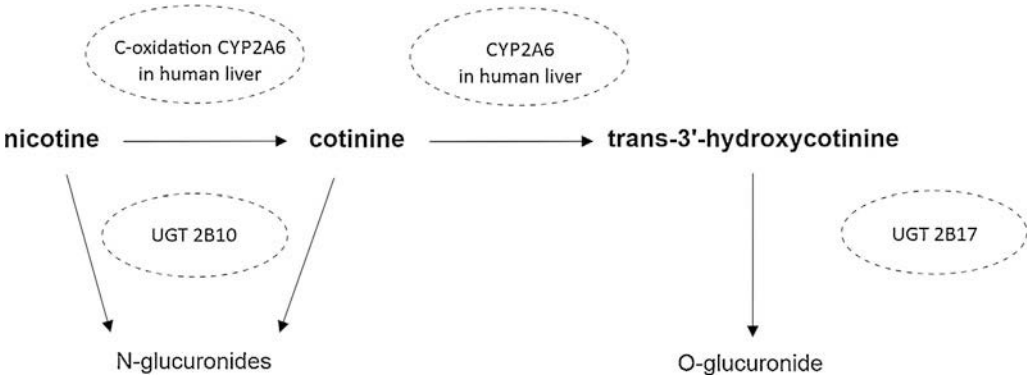

Fig. 1. Major metabolic pathway of nicotine in human [29,30] CYP2A6: Cytochrome P-450, enzyme isoform 2A6; UGT2B10: UDP-glucuronosyltransferase, enzyme isoform 2B10. Nicotine Metabolite Ratio (NMR) is a relation of *trans*-3′-hydroxycotinine to cotinine.

elimination [4,5]. Moreover, the clinical significance of the rate of nicotine metabolism has been demonstrated by its ability to predict the exposure level to tobacco-derived carcinogens in smokers [6].

The main source of variations in nicotine metabolism are genotype and environmental influences on CYP2A6 activity. According to the phenotype activity of that enzyme we can distinguish smokers as slower and faster metabolizers. Nicotine has a shorter elimination half-life (100–150 min) than the main metabolites: cotinine (770–1130 min) [7]. Nicotine and its metabolite cotinine are metabolized by the same enzyme. The measurement of molar ratio of two major nicotine metabolites: 3-HC/cotinine has been shown to be a phenotypic marker of CYP2A6 activity. It is commonly referred as to nicotine metabolite ratio (NMR). The specificity of this marker has been confirmed in numerous studies showing subjects with inactive CYP2A6 producing no 3-HC [8]. The ratio of 3HC to cotinine provides a stable measure of individual differences in nicotine metabolism. Moreover, NMR has been also shown to be independent of time since last cigarette [6]. The other parameter which indirectly describes the speed of nicotine metabolism is its clearance. It has been shown that nicotine clearance is highly correlated with NMR [9].

CYP2A6 allele frequencies vary substantially among the racial/ethnic groups. On average, whites metabolize nicotine faster than Blacks [10], Chinese-Americans and Asians [1,11]. Black smokers demonstrate a slower metabolic clearance of nicotine. There are also differences in nicotine metabolism between male and female smokers. In general, the clearances of nicotine and cotinine are 13 and 26% higher in women than in men [12].

The administration of estrogens may accelerate the clearance of nicotine and cotinine by as much as 30 and 33%, respectively, compared with women not using oral contraceptives [12,13]. Pregnancy has also an inducing effect in nicotine and cotinine clearance, however the clearance of cotinine is increased more than the clearance of nicotine [14]. In summary, the changes in nicotine and clearance is related to: 1) the levels of sex hormones; 2) hormonal estrogen replacement therapy; and 3) pregnancy. These effects are consistent with observed increased activity of CYP2A6 with sex hormones [7].

The other change in estrogen levels in women during lifetime is menopause. It refers to the permanent cessation of menses, resulting from the loss of ovarian follicular function [16]. If estrogens can induce CYP2A6 activity, it seems to be important what happened with nicotine metabolism ratio after the age of menopause. In Polish women population the overall median age at natural menopause is 51 years old [17].

Objectives

The aim of our work was to measure two major nicotine metabolites in the convenience sample of Polish smokers and estimate NMR distribution and its variation between women and men, taking into consideration women at age before and after the median age at natural menopause in Polish population.

Materials and methods

Study population

Urine samples were collected during the study assessing the relationship between smoking topography and tobacco biomarkers among daily smokers recruited in Silesia region in Poland as described previously [18]. The cross-sectional study included 187 subjects. All subjects were White with mean age of 36.3 ± 13.8 years and 42% of participants were male. Pregnancy was one of the excluding criteria for this study. In the group of women with age below 51 years (N = 78), average age was 31.5 ± 10.6 years (mean ± SD). In the rest of the population average age was 40.0 ± 14.8 years (mean ± SD), there were 63 of men with age below 51 years (30.2 ± 9.0), 26 of women with age more or equal 51 years (55.5 ± 5.7) and 13 of men with age more or equal 51 years (56.2 ± 5.6)

Analytical chemistry

The levels of nicotine metabolites: cotinine and 3-HC (both free and total forms) were analyzed by LC–MS/MS using liquid–liquid extraction with the use of similar method to Jacob et al. [9]. LC–MS/MS analyses were carried out with a Thermo Surveyor interfaced to a Thermo-Finnigan TSQ Quantum Ultra triple-stage quadrupole mass spectrometer. Separation of nicotine metabolites was performed using a 4.6 mm × 150 mm Phenomenex Synergi Polar RP column (4 mm) fitted with a Phenomenex Polar-RP guard column, 4 mm LX 3.0 ID. The limit of quantitation was 0.2 ng/mL for both cotinine and 3-HC. Urine samples were assayed before and after deconjugation with a glucuronidase enzyme (Sigma-Aldrich, USA). The concentration of unconjugated nicotine metabolites represents free cotinine and 3-HC, whereas the concentration after deconjugation represents sum of free and conjugated metabolite. Nicotine Metabolite Ratio (NMR) was calculated as a ratio of total 3-HC in nmol/g of creatinine to free cotinine concentration in nmol/g of creatinine, because 3-HC is created only from free cotinine (Fig. 1).

Table 1
Mean levels of nicotine metabolites (nmol/mg creatinine) and nicotine metabolites ratio among Polish smokers.

Group	BIOMARKER		GM [95%CI]	Median	IQR
Full sample N = 180	Cotinine [nmol/mg creatinine]	FREE	5.5 [4.7, 6.4]	9.6	3.5, 12.0
		TOTAL	14.6 [12.6, 17.0]	17.6	9.7, 27.8
	3-hydroxycotinine [nmol/mg creatinine]	FREE	20.6 [17.4, 24.4]	27.2	14.8, 39.3
		TOTAL	25.6 [21.6, 30.3]	33.9	18.8, 51.7
	nicotine metabolite ratio		4.8 [4.4, 5.2]	4.8	3.4, 7.3
Women under 51 years old N = 78	Cotinine [nmol/mg creatinine]	FREE	4.5 [3.5, 5.8]	5.3	2.9, 9.0
		TOTAL	13.2 [10.3, 17.0]	15.1	8.9, 25.9
	3-hydroxycotinine [nmol/mg creatinine]	FREE	21.0 [16.2, 27.2]	27.0	14.8, 38.1
		TOTAL	25.6[19.7, 33.2]	33.7	18.2, 50.5
	nicotine metabolite ratio		5.7 [5.0, 6.5]	6.4	4.1, 8.8
Excluding women under 51 years old N = 102	Cotinine [nmol/mg creatinine]	FREE	6.2 [5.1, 7.5]	6.4	3.7, 12.4
		TOTAL	15.1 [12.5, 18.4]	19.2	10.1, 29.2
	3-hydroxycotinine [nmol/mg creatinine]	FREE	19.5 [15.4, 24.7]	26.0	14.5, 38.9
		TOTAL	24.4 [19.3, 30.9]	31.6	18.8, 48.8
	nicotine metabolite ratio		4.2 [3.7, 4.7]	4.3	2.8, 6.4

GM – geometric mean; CI – confidence interval; IQR – inter-quartile range (25%–75%).

Statistical analysis

We generated histograms illustrating distribution of cotinine, 3-HC and NMR in the study population. We tested the results for normality with the Shapiro-Wilk test. The distribution of free and total cotinine, 3-HC and NMR was found to be log-normal distributed, so median and inter-quartile range (IQR) was calculated to illustrate average trends of these parameters. All statistical tests were two sided with a significance level of $\alpha = 0.05$. Study participants were divided into four groups according to gender and age (at or above 51 years *versus* below 51 years). Because the data were not normally distributed, data were Box-Cox transformed with grouping variables (age groups and sex) resulting in data with no shift and lambda = 0.44. After testing for normality in each of the transformed groups (Shapiro-Wilk test) and equality of variances (Levene's test), to assure uniformity in the NMR results in the total population excluding women below 51 years old, ANOVA and Scheffe *post-hoc* analysis was conducted to determine whether there were differences in NMR between men below and above 51 years old and women above 51 years old. Next, a *t*-test was used to compare NMR between women below 51 years old (N = 78) and the rest of population (N = 102; women above 51 years old and men in both age groups). All statistical analyses were performed with Statistica 10.0 (Statsoft Inc., USA)

Results

The average urine levels of free cotinine, total cotinine, free 3-HC and total 3-HC in general population were 9.6 (IQR 3.5–12.0); 17.6 (IQR 9.7–27.8); 27.2 (IQR 14.8–39.3); 33.9 (IQR 18.8–51.7) nmol/g of creatinine, respectively (Table 1). For all female smokers the average levels of free cotinine, total cotinine, free 3-HC and total 3-HC were 5.8 (IQR 3.4–10.8); 16.5 (IQR 9.6–29.2); 27.2 (IQR 15.0–40.0); and 33.7 (IQR 18.6–52.2) nmol/g of creatinine, respectively (Table 1). For male smokers (42% of examined population) the average levels of free cotinine, total cotinine, free 3-HC and total 3-HC were 5.9 (IQR 3.3–12.8); 16.4 (IQR 9.0–25.8); 25.7 (IQR 12.0–37.4); 29.9 (IQR 17.2–46.6) nmol/g of creatinine, respectively (Table 1).

Nicotine Metabolite Ratio (NMR) in whole population was 4.8 (IQR 3.4–7.3). The distribution of NMR in a whole population is presented on Fig. 2. NMR was higher among female than male smokers (5.2 (IQR 3.8–8.3) *vs.* 4.5 (IQR 2.7–6.9); $p = 0.04$; Table 1).

A *post hoc* analysis (Sheffe test) showed no statistical differences between the groups of women with age more or equal 51, men with age more or equal 51 and men with age below 51 ($p = 0.55$). According to that, we combined these groups into one for the next stage of analysis. Statistical analysis revealed a significant difference between the group of women below 51 year of life, and the rest of the population (*t*-test, $p = 0.0002$).

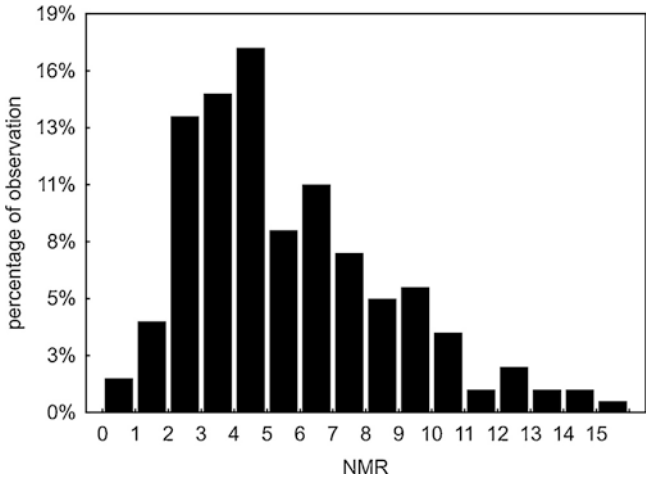

Fig. 2. Distribution of NMR withouth transformation in examined population (n = 180, 58% female).

Discussion

Study implications

Estimation of NMR is a practical non-invasive tool for assessing the rate of nicotine metabolism in humans. NMR is a phenotype marker of CYP2A6 activity, which demonstrates, for example, differences between metabolism in women and men [10]. NMR measured in saliva or plasma after administration of nicotine (by tobacco smoke) is correlated with oral clearance of nicotine [8]. This rate of nicotine metabolism is important when we consider the time over which nicotine acts on receptors in brain. This time may vary dependent of the activity of CYP2A6 [19]. It may be the reason for different smoking behaviors among smokers. It has been shown that NMR has predictive validity as a marker for successful quitting with nicotine replacement therapy (NRT) [2]. NMR is a good indicator of nicotine metabolism among those smokers who smoke systematically, have a constant smoking habits, and have reached the steady state level of 3-HC, whereas among people who smoke occasionally, the ratio may be underestimated [20].

The prevalence of *CYP2A6* gene variants associated with slow nicotine metabolism is low in Caucasian smokers [3,10,21]. Our

results suggest that women before menopause differ in phenotype of nicotine metabolism, due to other factors. The most likely element is higher estrogen levels.

We found significant differences in NMR among women and men consistent with previous studies by Johnstone et al. and Swan et al. who showed greater CYP2A6 activity and faster metabolism of nicotine in women [10,22]. In our study, NMR in women was significantly higher in age below 51 in comparison to the rest of the population. Lower NMR in women above 51 years is most likely due to the declining estrogen levels around this age (menopause).

Accelerated nicotine metabolism appears to be a result of estrogens, which level decreases with age.

Clinical implication of study findings

Variations in nicotine metabolism might have important clinical implications during smoking cessation with nicotine replacement therapy. Slow metabolizers have higher nicotine plasma levels when using nicotine patch in comparison to normal metabolizers after using the same number of nicotine patches per week, while when using nicotine spray, both groups were achieving similar nicotine levels [3]. Studies suggest that same therapy may have different efficacy depending on the CYP2A6 activity. Smokers shown to be fast metabolizers by the ratio of 3-HC/cotinine have a lower rate of cessation success. That means that nicotine replacement therapy among these patients is not as effective as among poor metabolizers. Faster metabolizers seem to require higher doses of nicotine from NRT since it has been shown that doses such as 21 mg per patch, are not effective in this group [5]. Based on our results, it could be tentatively concluded that NRT with standard dosing may not be an effective way of smoking cessation therapy in younger women, and that higher doses may be necessary or treated with bupropion or varenicline [23]. Non-nicotine pharmacotherapy might also be more effective as women smoke more for non-nicotine reasons [24–26].

Study limitations

An important limitation of our study is that we did not survey women for the use of oral contraceptives, that according to Benowitz et al. [15] and Berlin et al. [27] may induce the metabolism of nicotine, but from the evidence presented in Rubinstein et al. [28] this influence seems to be inconclusive in adolescents. Also, menopause was not verified in the sample.

Future directions

Further studies are needed to evaluate if adjusting nicotine doses or selecting non-nicotine smoking cessation medications for rapid metabolizers in clinical practice in Poland based on NMR phenotyping can increase the successful rate in smoking cessation attempts.

Conclusions

Our results indicate faster nicotine metabolism among women younger than 51 years old in comparison to men in all ages and women older than 51 years. This is most likely due to the influence of estrogen which has been shown to induce nicotine metabolism. As NMR may be helpful in assessing initial classification of patients for smoking cessation therapies, findings from this study suggest that clinicians and pharmacists should consider adjusting nicotine dosing from NRT products among younger women.

Conflict of interest statement

Dr. Benowitz serves on advisory boards of several pharmaceutical companies that market smoking cessation medications. Dr. Goniewicz has received a research grant from and served as a member of advisory board to pharmaceutical companies that market smoking cessation medications. Dr. Sobczak accepted personal fees from the eSmoking Institute in Poznan, Poland, and non-financial support from Chic Group LTD, a manufacturer of electronic cigarettes in Poland, outside of the submitted work. Other authors have no conflicts to declare.

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