Toenail Nicotine Levels as a Biomarker of Tobacco Smoke Exposure[1]

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Abstract

Currently used biomarkers of tobacco smoke exposure have several disadvantages, including that they reflect short-term exposure and can therefore be affected by day-to-day variations. The aim of this study was to assess the validity of toenail nicotine levels as a biomarker of exposure to tobacco smoke for use in epidemiological studies. Toenails were collected in 1982 from 62,641 women participating in the Nurses' Health Study, whereas questionnaire data at that time provided information on active and passive smoke exposure. A stratified random sample of stored toenails from 106 women were selected according to their reported exposure category. Toenails were analyzed for nicotine levels by high-performance liquid chromatography. Toenail nicotine levels differed significantly according to tobacco smoke exposure ($P < 0.0001$). Among nonactive smokers, there was a significant difference in toenail nicotine levels between passive smokers (mean = 0.28 ng/mg) and nonexposed women (mean = 0.08 ng/mg; $P = 0.0006$). Among active smokers, there was also a significant difference ($P = 0.04$) in mean nicotine levels according to categories of cigarettes smoked (means for smokers of 1–14, 15–24, and 25 or more cigarettes/day were 0.94, 1.81, and 2.40 ng/mg). An overlap of the distribution of nicotine levels among passive and active smokers was found. Among all women, the correlation between nail nicotine levels and smoking exposure categories was $r = 0.80$ ($P < 0.0001$). The results of this study indicate that toenail nicotine level measurement is a valid biomarker for assessment of active and passive exposure to tobacco smoke. Nail nicotine levels may reflect aspects of active and passive exposure not captured by standard questionnaires and, thus, have the potential to provide better assessment of associations with health risk.

Introduction

Active tobacco smoking is a proven cause of lung and other cancers, cardiovascular diseases, respiratory illnesses, and other conditions (1). Secondhand smoke (also known as ETS[3] or passive smoking) exposure has been implicated in these same diseases (2). However, for passive smoking, almost all studies have relied on questionnaire reporting of exposure to an actively smoking spouse or from estimations of the number of cigarettes smoked around nonsmokers (3). The magnitude of the risk because of ETS could be greatly attenuated because of measurement error (4, 5). For example, typical questionnaires may not have accurately accounted for other regular sources of exposure outside the home or from social events with high levels of exposure. Furthermore, questionnaires are limited in their ability to account for variability in smoking habits of the spouse and the type of ventilation and are subject to recall and reporting bias (6–8).

Biomarkers have had limited use in studies assessing ETS or active smoking in relation to risk of chronic diseases. This is mainly attributed to the short duration represented by exposure measurements, which are at most 2–3 days for urine cotinine levels. Human toenails grow at a rate of 1 cm every 9–12 months (9), and their use could reflect relatively longer cumulative exposure period and thus minimize the effect of exposure variation. If nicotine (the main constituent of tobacco smoke that is measured as a marker of the other constituents of tobacco smoke) is accurately measured in toenails, which are presumed relatively free of external contamination, this could provide an indicator of exposure to tobacco smoke with advantages over existing biomarkers. Furthermore, toenails are easily collected and stored. Verghese *et al.* (10) in 1990 described what they termed a curious physical sign, "harlequin nail," which was a distinct line of demarcation in the nails showing when the patient had stopped smoking. To our knowledge, no other studies have examined the use of nail nicotine levels for assessment of chronic exposure to tobacco. In this study, we aimed to assess the validity of toenail nicotine levels as a biomarker of exposure to tobacco smoke for future use in epidemiological studies of cancer and other diseases.

Materials and Methods

Study Population. The Nurses' Health Study was established in 1976 when 121,700 United States female registered nurses ages 30–55 years and residing in one of 11 states completed a mailed questionnaire regarding medical history and lifestyle factors. This information has been updated every 2 years. In 1982, participants were asked to collect toenail clippings from their 10 toes and send them in a provided envelop. Toenails were collected from 62,641 women. Cancer patients (other than nonmelanoma skin cancer) were excluded because of the un-

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[3] The abbreviations used are: ETS, environmental tobacco smoke; HPLC, high-performance liquid chromatography;

predictable effect of cancer and its treatment on the incorporation of nicotine into toenails. Women whose nail samples have been used in earlier studies or women who failed to provide information on smoking history or ETS exposure history were also excluded. Therefore, the nail samples from 55,675 women were available for this study.

Smoking History. The validity of the toenail biomarker was assessed by comparison of toenail nicotine levels with self-reported smoking history, including passive smoking. At the beginning of the Nurses' Health Study cohort in 1976, participants were asked about their smoking status. The smoking status question was repeated in each 2-year cycle. If they were active smokers, they were asked how many cigarettes they smoked per day and according to the following categories: 1–4; 5–14; 15–24; 25–34; 35–44; or ≥45 cigarettes/day. For this analyses, we used the smoking status from the 1982 questionnaire. Passive smoking was recorded in the 1982 questionnaire according to the following questions: "are you currently exposed to cigarette smoke from other people at work or home?" [choices of answers: at home (no, occasionally, regularly), at work (no, occasionally, regularly)]. These questions allowed us to classify each of the subjects with regard to current ETS (both at home and at work) and active smoking status (number of cigarettes smoked). Using the above information, we used the following main exposure variables for analyses: never smokers or past smokers who had quit for ≥4 years; passive smokers who were not active smokers; active smokers without passive exposure; and active smokers who were also exposed to passive smoking. The passive smoke variable was dichotomized as not exposed to ETS or exposed at either home or work. Finally, active smoking was categorized according to the number of cigarettes smoked: 0; 1–14; 15–24; or ≥25 cigarettes/day.

Study Sample. A random sample of women with available toenails were selected within the following main smoking subcategories in 1982: 25 never smokers or past smokers with no ETS exposure; 29 passive smokers who were not active smokers; 26 active smokers who were not exposed to ETS; and 26 active smokers who were also exposed to ETS. Past smokers in 1982 who had quit before the 1980 questionnaire were included with the passive and never-exposed groups. Because toenails measure up to 1 year of past exposure and because the toenails were collected in 1982, we wanted to minimize any possibility of active smoking among our nonactive smoking group and thus only included past smokers who had stopped smoking for ≥4 years. Those who reported not being current smokers in the 1980 questionnaire did not smoke in 1979 and 1978; we therefore assumed that they had quit for ≥4 years before toenail collection. As a result, a total of 106 samples was used for the analyses of this study.

Protocol for Nail Collection and Laboratory Analyses. From preliminary laboratory analysis, we anticipated that three medium-sized toenail clippings (10–30 mg) would be enough for the purpose of the analysis. The laboratory technician was blinded to the smoking category of the samples.

The nail analysis was carried out at the Wellington Hospital biochemistry laboratory, using HPLC with electrochemical detection specifically developed for analysis of nicotine levels in hair (11). Sample preparation and analyses in the laboratory were similar to hair samples and involved washing the samples with dichloromethane after cutting and weighing them. The nails were then digested at 50C° overnight in 2 ml of 1.0 mol/l NaOH before passing them through the HPLC column (for additional details on the assay, see Ref. 11). A typical chromatogram of toenail nicotine levels is shown in Fig. 1. If there was enough toenail mass for an individual, samples were run in duplicates to reduce random error (40 samples were run in duplicates). The method has been shown to have high sensitivity (lower detection level of <0.01 ng/mg nail) and high within batch reproducibility (coefficient of variation % <10%, *i.e.,* the variability between two samples from the same individual analyzed by HPLC during one group of samples).

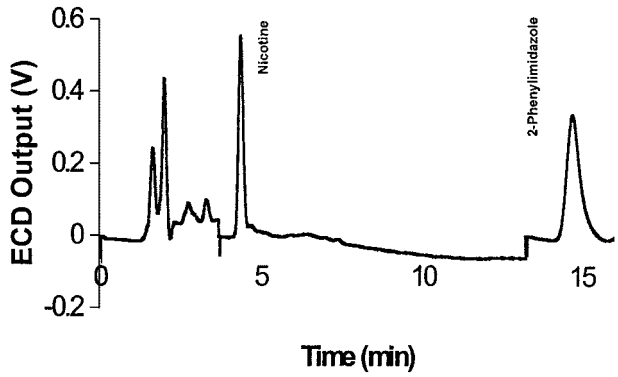

Fig. 1. A typical chromatogram from an HPLC reading of nicotine levels in toenail (1.7 ng/mg nail) compared with 2-phenylimidazole as the internal standard.

Data Analyses. The Kruskal-Wallis nonparametric test for nonnormal data were used to compare toenail nicotine levels according to the reported smoking habit. Nicotine levels were assessed by reported ETS exposure separately from home and from work and by categories of cigarettes per day among active smokers. Spearman's correlation coefficient was also used to assess the association between different ranked exposure levels and nicotine levels in toenails.

Regression analysis [the REG procedure in SAS (12)] was used to assess the relation between smoking categories and nail nicotine level, adjusting for age. The regression analyses also allowed assessment of the independent contributions of active and passive exposure to toenail nicotine levels, after adjusting for age. Age may be related to rate of growth of nails and thus possibly to nicotine levels in toenails. These models were repeated among the nonactive smokers only. Also, we assessed the independent contributions of ETS to nicotine levels among active smokers by including indicator variables for passive smoking and number of cigarettes smoked in a model restricted to active smokers.

Results

No appreciable difference in age or body mass index was found among the different exposure groups (Table 1). Among passive smokers, all were exposed at work but only 34% were exposed at home. Nail nicotine levels were lowest among nonexposed participants and highest among active smokers who were also exposed to ETS. There was a significant difference in toenail nicotine levels according to the four main categories of exposure ($\chi^2 = 69.8$, $P < 0.0001$; see Table 1). Among women who were not active smokers, there was a significant difference in toenail nicotine levels between nonexposed women and ETS-exposed women (mean = 0.08 ng/mg for nonsmokers not exposed to ETS and 0.28 ng/mg for nonsmokers exposed to ETS; $P = 0.0006$). There was also a statistically significant difference in nicotine levels according to categories of cigarettes smoked by active smokers (means for smokers of 1–14,

Table 1 Characteristics of 106 women according to their categories of exposure

Exposure	*n*	Mean nicotine (ng/mg)	Mean age (yr)	Body mass index	Exposed at home[a]	Exposed at work[a]
0 (no exposure)	25	0.08	48	23.6	0	0
1 (passive)	29	0.28	47	24.8	34%	100%
2 (active)	25	1.71	49	22.6	0	0
3 (active & passive)	26	2.18	48	22.8	77%	88%

[a] Passive smoking at home or work (no exposure *versus* occasionally or regularly exposed).

Table 2 Regression model results (R^2, β, and *P*s for each variable in the model) for different combinations of independent variables in relation to the log toenail nicotine levels (ng/mg hair) as the dependent variable

	n^a	Main:[b] β (*P*)	Active:[c] β (*P*)	Passive: β (*P*)
Model 1, $R^2 = 0.62$	104	1.15 (<0.0001)		
Model 2, $R^2 = 0.64$	102		1.05 (<0.0001)	0.69 (0.0004)
Model 3, $R^2 = 0.65$	102		0.99 (<0.0001)	0.41 (0.005) home
				0.15 (0.24) work
Model 4, $R^2 = 0.59$	103		1.05 (<0.0001)	
Model 5,[d] $R^2 = 0.20$	53			0.93 (0.0005)
Model 6,[d] $R^2 = 0.27$	53			0.54 (0.008) home
				0.38 (0.02) work
Model 7,[e] $R^2 = 0.16$	48		0.51 (0.007)	0.46 (0.08)
Model 8,[e] $R^2 = 0.12$	48		0.52 (0.007)	

[a] Some had missing information.
[b] This refers to the four main exposure categories of the study (never exposed, passively exposed, actively exposed, and actively and positively exposed).
[c] This represents the categories of active cigarette smoking.
[d] Excluding active smokers.
[e] Excluding nonactive smokers.

15–24, and ≥25 cigarettes/day were 0.94 ng/mg, 1.81 ng/mg, and 2.40 ng/mg, respectively; $P = 0.04$). We also assessed the ability of toenail nicotine levels to predict long-term exposure by comparing the levels in 1982 with reported smoking status [never smokers ($n = 54$), current smoker ($n = 47$)] in 1976 and found a statistically significant difference between the two groups (mean nicotine levels of never smokers = 0.19 ng/mg and for current smokers = 2.01 ng/mg; $P = 0.001$).

The Spearman correlation between nicotine levels and overall increasing level of smoking exposure was $r = 0.82$ ($P < 0.0001$). For the four main exposure categories, the correlation was $r = 0.80$ ($P < 0.0001$). Adjusting for the number of cigarettes actively smoked attenuated the correlation between nicotine levels and reported exposure to ETS among combined active and nonactive smokers, but this was still statistically significant ($r = 0.38$; $P < 0.0001$).

The results of regression analyses are summarized in Table 2. We included the R^2 values to assess the fit of each model, and the β coefficient and their significance for the contribution of each independent variable to the model. The models that contained the four main exposure categories, the passive and active exposure variables, or just the active variable explained most of the variability of nicotine levels in toenails (models 1–4 in Table 2). The nicotine levels more than doubled (216% change) for each category of the main exposure variable (model 1). The percentage change for the active smoking and passive smoking variables was 186 and 99% (in model 2).

In the model restricted to nonactive smokers (model 5), passive exposure was statistically significant, although R^2 was reduced to 20%. When categorizing passive smoking exposure as two separate variables according to the place of exposure, the contribution of exposure from home was higher (as shown by the b estimate value) compared with the lower contribution of exposure from work (model 6). Among active smokers only, the R^2 was 16% for the model, including the number of cigarettes smoked and reported passive smoke exposure (the term for passive smoking among active smokers just failed to attain statistical significance, $P = 0.08$; model 7), and the R^2 value was further attenuated when the passive smoking variable was excluded (model 8).

The distributions of nicotine levels are shown according to the smoking categories in Fig. 2. There were large differences between active and nonactive smokers and between nonsmokers not exposed to ETS and nonsmokers exposed to ETS. Although the difference in the nicotine levels between smokers and nonsmokers is clearly shown by the figure, there was substantial overlap between women only exposed to ETS and active smokers not exposed to ETS.

Discussion

This cross-sectional analyses of tobacco exposure during the same period as toenail collection indicates that toenail nicotine levels can be informative biomarkers for the assessment of exposure to tobacco smoke. Women who were not active smokers who were exposed to ETS had higher nicotine levels than nonexposed individuals, but both categories of nonsmokers had much lower nicotine levels than active smokers. Nicotine levels of passive nonactive smokers overlapped with those of active smokers, which supports the hypothesis that exposure to passive smoking can be a significant source of exposure that is usually not accounted for in studies of adverse health effects of active smoking based on questionnaire measurements only.

The limitations of validating a new biomarker with an imperfect one such as questionnaire in this case are well known. Although the two measures were highly correlated, nail nicotine was less strongly correlated with questionnaire reports of passive smoking. Given the many factors that affect ETS ex-

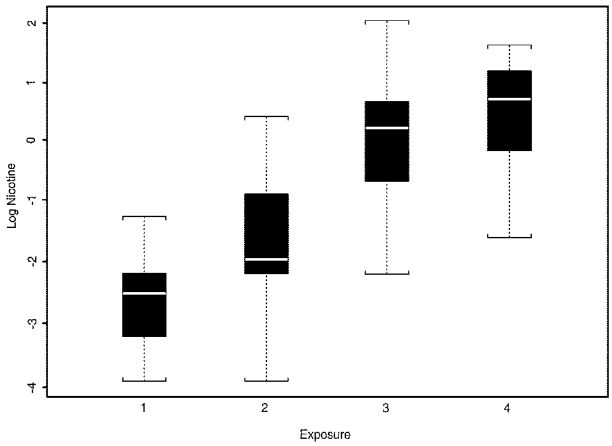

Fig. 2. Box plot distribution (unadjusted) of log (normal) hair nicotine levels according to their reported exposure categories. 1 = no exposure; 2 = nonsmokers exposed to ETS; 3 = active smokers not exposed to ETS; 4 = active smokers exposed to ETS. *Whiskers* are 99 percentiles, *upper edge of box* are 75 percentiles, and *lower edges* are 25 percentiles; the *white middle bar* is the median.

posure measurement (such as room ventilation, number and intensity of sources of exposure, and reporting bias), it is not surprising to find that questionnaire reports did not more strongly explain the levels of toenail nicotine levels among passive smokers. The large variation in nicotine levels among passive smokers suggests there is additional information conveyed by nail levels than by questionnaires on passive smoking alone. However, because of the absence of an independent gold standard for measurement of ETS, it is difficult to conclude which measure is better. One important validity criteria of a biomarker is its ability to predict illness in a dose-dependent manner. Therefore, using this biomarker, additional studies will be needed to address the relative validity of toenail nicotine levels as compared with questionnaire assessments and passive smoking in predicting diseases such as lung cancer and cardiovascular diseases.

Although this study had enough power to detect a difference between the four main exposure categories of our interest: never exposed, passively exposed, actively exposed, and actively and passively exposed as shown by our results, the small sample size did not allow adequate assessment of the subcategories of passive smoking (regular or occasional and at work or at home) or the number of cigarettes smoked by active smokers. Also, future studies with sufficient number of toenails samples to better assess the degree of overlap between passive and active smokers are needed. The small sample size, the mostly white population of the nurses population, and the lack of genetic data also limited our ability to assess the influence of race and genetics on nail nicotine levels.

To our knowledge, no other study has examined the use of this biomarker for quantitative assessment of exposure to tobacco smoke. A similar marker, hair nicotine, has been recently found to be an informative indicator of exposure to tobacco smoke (13–15) and better correlated with exposure history than the widely used urine cotinine biomarker (16, 17). This may be attributable to its relatively long-term exposure assessment ability (18, 19), which is important in overcoming the day-to-day short-term tobacco exposure variation, especially in regards to ETS. Human nails are formed of the same keratinous tissue of hair and are therefore expected to have the same qualities and advantages of the hair biomarker. Furthermore, toenails grow at a much slower rate than hair (1 cm/9–12 months; Ref. 9) and are less vulnerable to environmental contamination with nicotine. The significance of the combination of low cost and high sensitivity of the HPLC method, makes this biomarker (and hair nicotine), a cost-effective epidemiological tool.

Home exposure to ETS is known to be the major source of indoor pollution (20). This is supported by our findings that passive exposure at home was a significant independent variable. Although more people were exposed at work, the amount of exposure at home seems to be more than at work (models 3 and 6) or home exposure may be better reported.

Our study suggests a possible contribution of ETS exposure to nail nicotine levels among active smokers as observed in regression analyses (models 7 and 8 in Table 2). There was approximately a 4% contribution of ETS to active smokers nicotine levels. Accounting for ETS exposure among active smokers may increase the currently known risk ratios of tobacco related adverse health effects because failure to do so can contribute to nondifferential measurement error and attenuation of risk ratios toward the null. Also, the overlap of nicotine levels between passive and active smokers (Fig. 2) suggests that the effect of active smoking may often be underestimated as the comparison group may include persons with significant passive exposure.

In conclusion, in this study, large differences in toenail nicotine levels were seen among women categorized by reported exposure history. The use of toenail nicotine biomarker is a novel way to measure active and passive exposure to tobacco smoke objectively, which has in the past been largely measured by self-reported questionnaires or short-term biomarker measurements. Because nail nicotine levels may reflect sources of active and passive exposure not captured by standard questionnaire (as supported by overlap in nicotine levels between passive and active smokers), they may provide better assessment of associations with health risk as a result of a more precise measure of long-term exposure. The issue of passive smoking among active smokers is an important but inadequately addressed topic in tobacco research. Larger studies are needed to evaluate further the use of this biomarker in epidemiological studies.

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