Measurement of Tobacco Smoke Exposure: Comparison of Toenail Nicotine Biomarkers and Self-Reports

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Abstract

Background: **Accurate measurement tools of exposure for use in large epidemiologic studies are lacking. Biomarkers of tobacco exposure provide additional advantages to self-reports and there is a need to further develop and validate them. The objective is to compare toenail nicotine levels, a novel biomarker of tobacco exposure, with self-reports of tobacco exposure from a large cohort study.**

Methods: **In this cross-sectional analysis, toenail samples were collected from 2,485 women participating in the Nurses' Health Study in 1982. Detailed self-reports of smoking habits and reported exposure to secondhand smoke (SHS) were collected from these women near the time of toenail collection. The toenail samples were analyzed by a high-performance liquid chromatography method for measuring nicotine.**

Results: **The 5 to 95 percentile range of toenail nicotine was from 0.06 to 4.06 ng/mg toenail and the median level was 0.21 ng/mg. There was a significant difference in toenail nicotine levels according to reported smoking status (the median level for nonsmokers with no SHS was 0.10 ng/mg, the median level for nonsmokers with SHS was 0.14 ng/mg, and the median level for active smokers was 1.77 ng/mg). However there was considerable overlap in nicotine levels according to reported smoking status. Toenail nicotine level was strongly associated with reported smoking level (Spearman r = 0.63), but there was no complete concordance, suggesting that the two methods are measuring different aspects of the same exposure.**

Conclusion: **Our findings show that toenail nicotine levels capture the overall burden of tobacco smoke exposure and provide additional information on exposure not captured by reported history.** (Cancer Epidemiol Biomarkers Prev 2008;17(5):1255–61)

Introduction

Assessment of exposure to tobacco smoke, and especially secondhand smoke (SHS), continues to be a challenging area that needs further development to better assess existing and new tobacco-related health risks (1). The lack of accurate and reliable methods for measurement of long-term exposure to tobacco smoke leads to measurement error, which results in an underestimation of the health risks (2). Questionnaires are still the most common method of measuring exposure to tobacco smoke, although biomarkers and other environmental and personal monitors are being frequently used (3-6). Nevertheless, there is no ''gold standard'' method for measurement of the overall exposure to tobacco smoke. Some believe that the number of cigarettes per day is an accurate measure of exposure among active smokers. However, it has been shown that variation in smoking habits, such as the length or frequency of puffs (known as smoking topography), can lead to different levels of exposure even when smoking the same amount of cigarettes (7). Furthermore, in certain situations, such as during pregnancy or among adolescents, smokers may deliberately or unconsciously underreport smoking status or the number of cigarettes smoked (3, 8-11) because of growing awareness of health risks and social rejection of smoking. Another important source of variability of exposure to tobacco smoke that cannot be quantified by self-report of smoking are differences in metabolism of tobacco, which varies by race, gender, and age, and leads to different levels of internal exposure from smoking a similar number of cigarettes (12).

Most studies of active smokers never report on SHS exposure (also known as passive smoking), which is not captured by reporting the number of cigarettes smoked per day. SHS is a complex and dynamic exposure that is affected by the intensity of the source of active smoking, the size of the space where exposure took place, the ventilation in such a space, and the frequency and duration of exposure (6), which are all difficult to quantify precisely. Questionnaires for SHS measurement may introduce random error and bias because most subjects fail to recall all their exposures to SHS and do not objectively quantify that exposure.

Biomarkers of tobacco smoke exposure offer the advantage of measuring the internal dose of exposure and therefore overcoming the subjectivity of reporting. Some of the earlier biomarkers, such as carbon monoxide in breath, serum cotinine and nicotine levels, and salivary cotinine, provide short-term measurement of tobacco exposure but fail to overcome the problem of daily variability. This is also a major problem for the more widely used biomarkers such as urine cotinine

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(13-15). Longer-term biomarkers are more relevant to chronic diseases that are associated with tobacco exposure, such as cardiovascular diseases and cancer, because these biomarkers would minimize the day-to-day variability of the exposure because of their longer half-life. Recently, promising results from two of these biomarkers have been reported from epidemiologic population studies, the nicotine hair measure (5, 16) and the toenail nicotine measure (17, 18). Based on pilot data, toenail nicotine levels appear to be promising in terms of nicotine specificity and sensitivity and capability of measuring up to 12 months of past exposure (17). Nicotine is incorporated into toenails as the toenails grow from the bed of the toenail. Because of the slow growth rate of toenails, the cut toenail clippings represent exposure in the last few months. This has the main advantage of representing longer duration of exposure and therefore less variation than short-term biomarkers of salivary, serum, or urine nicotine and cotinine. In the previous pilot study, there were only 106 participants, which limited detailed analyses and interpretation. In this article, we analyze nicotine levels in toenail samples collected from 2,485 women participating in the Nurses' Health Study (NHS) and compare these measurements with their reported active smoking and SHS exposure.

Materials and Methods

The study population is part of the NHS, which was established in 1976 when 121,700 U.S. female registered nurses ages 30 to 55 years and residing in 1 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 the provided envelop.

Population and Study Design. From the NHS study population, 62,641 women provided toenails in 1982 and were free from stroke, heart disease, and cancer (other than nonmelanoma skin cancer); cancer patients were excluded due to the unpredictable effect of disease and its treatment on the incorporation of nicotine into toenails. Also, women whose nail samples had been used in earlier studies and those who failed to provide information on smoking history or SHS exposure history were excluded.

This was a cross-sectional analysis involving toenail measurements of tobacco smoke exposure in 1982. The toenails used in analyses were from a nested case-control study within NHS investigating the association between tobacco smoke and coronary heart disease (19).

Tobacco Smoke Exposure Variables. Women who reported being current smokers in the 1982 questionnaires were considered as active smokers and were further categorized according to their reported number of cigarettes smoked per day as follows; 1 to 4, 5 to 14, 15 to 24, 25 to 34, 35 to 44, or ≥45 cigarettes per day. Past smokers were those who reported not smoking at the time of toenail collection in 1982 but were previously smokers. SHS exposure was reported in 1982 as follows: ''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)]. Never exposed are those who were never smokers up to the 1982 questionnaire and reportedly not being exposed to tobacco smoke from others in 1982.

Protocol for Nail Collection and Laboratory Assay. Toenails were selected from the stored NHS samples for laboratory analyses. The toenail samples could not have been used for other studies. Selection was made by identifying the ID number of each woman who developed coronary heart disease after the return of the toenail samples in 1982 and two randomly selected women who did not have a diagnosis of coronary heart disease at the time and were matched for the date of return of the nail sample and age within a year.

The nail analyses were carried out at the Wellington Hospital biochemistry laboratory using the high-performance liquid chromatography with electrochemical detection method (20). The samples were blinded to the laboratory officer analyzing them. Sample preparation in the laboratory involved washing the nails with dichloromethane after cutting them up into small pieces and weighing five clippings, including one from the large toe, out of the 10 clippings provided by participants. Nicotine levels from toenails are calculated as nanogram of nicotine per milligram of toenail and therefore overcoming any difference in length or thickness of toenails. The nails were then digested at 50°C overnight in 2 mL of 1.0 mol/L NaOH before running them through the high-performance liquid chromatography column. The % coefficient of variation for the assay was 12.6% when running samples in duplicates.

Data Analyses. Medians, ranges, and percentages were used to describe the characteristics of the population and the distribution of smoking-related variables. Because toenail nicotine levels were not normally distributed, the Kruskal-Wallis test for nonparametric data was used to compare toenail nicotine levels according to the reported smoking habits. Nicotine levels were analyzed according to reported smoking classified in several ways: smoking status (never smokers, past smokers, current smokers), smoke exposure (excluding past smokers: never smoked nonexposed, never smoked exposed to SHS, and active smoking), SHS exposure (no, yes), frequency of SHS exposure (no SHS exposure, occasional SHS exposure at the home or work, and frequent SHS exposure at either the home or work), and number of cigarettes smoked per day (0, 1-4, 5-14, 15-24, 25-34, ≥35). Finally, a composite smoking status variable was created as follows: never smokers, past smokers, current smokers who smoke 1 to 14 cigarettes per day, current smokers who smoke 15 to 24 cigarettes per day, current smokers who smoke 25 to 34 cigarettes per day, and current smokers who smoke ≥35 cigarettes per day.

Using the REG procedure in SAS, regression analyses were carried out to assess the variables for smoking exposure: age in years, body mass index (<21, 21-22.9, 23-24.9, 25-28.9, ≥29), physical activity (<1, 1-1.9, 2-3.9, 4-6.9, ≥7 h/wk of moderate to vigorous activity; ref. 21), alcohol intake (0, 0.1-4.9, 5-14.9, ≥15 g/d), presence of fungus on toenails (yes, no), and presence of nail polish on toenails (yes, no) as possible predictors

of the log-transformed toenail nicotine levels (toenail nicotine levels were log transformed to minimize the skewed distribution of this variable). Alcohol intake was not reported in 1982 when the toenails were collected. We therefore used the reported intake from 1980 with the assumption that the intake did not change substantially over the next 2 years. With the exception of age, the other covariates were excluded from the final models because none of them was a statistically significant predictor. The above regression analyses were repeated for each of the above smoking-related variables. We also included both the number of cigarettes and reported SHS exposure in the same model to assess if the SHS variable can significantly predict toenail nicotine levels over and above the prediction of the reported number of cigarettes smoked.

Finally, we examined the overlap between nicotine levels among SHS and light active smokers. To conduct this analysis, we first determined the lower 95% confidence boundary level of nail nicotine among active smokers and then assessed the percentage of women exposed to SHS above this level. We also used the median level among women smoking 1 to 4 cigarettes per day as the hypothetical cutoff for overlap with SHS.

Results

The median age for women in our study was 56 years, and their median body mass index was 24 kg/m^2. As shown in Table 1, in this population, 31% were current smokers and a similar percentage were never smokers who reported exposure to SHS. The 5 to 95 percentile range of toenail nicotine was from 0.06 to 4.06 ng/mg toenail and the median level was 0.21 ng/mg. For the subgroups of the ''smoke exposure,'' the median nicotine levels were 0.10 ng/mg for never smokers with no SHS, 0.14 ng/mg for never smokers with SHS, and 1.77 ng/mg for active smokers. The ranges for these categories overlapped with each other. The upper 25% levels for SHS overlapped with the lower 5% of active smokers, and upper 10% of SHS overlapped with the lower 50% of light active smokers (1-4 cigarettes per day; data not shown). Only 5% of the toenail samples were covered with nail polish, but 23% of the toenail samples showed evidence of fungus infection (Table 1). As noted earlier, these were not associated with nicotine levels.

Figure 1 presents the distribution of smoking exposure according to deciles of toenail nicotine levels. The active smokers were mostly in the 8th, 9th, and 10th deciles, whereas nonexposed never smokers were mainly in the first and second deciles. Although there was a significant difference among the three groups of women (Kruskal-Wallis $P < 0.0001$), never smokers exposed to SHS overlapped with the never exposed in their distribution and also with the active smokers. Slightly more than 5% of the women exposed only to SHS were in the 8th decile of toenail nicotine levels, suggesting high SHS exposure. In Figure 2, the median and 25 to 75 percentiles are presented for the categories according to the number of cigarettes, where 0 cigarettes represented nonsmokers. There was a significant difference ($P < 0.0001$) among the categories, but the figure shows major overlap in nicotine levels for the three highest categories of reported cigarettes smoked by active smokers (15-24, 25-34, and $\geq$35 cigarettes per day).

Table 2 presents the association between categories of smoking status (nonsmokers, past smokers, current smokers who smoke 1-14 cigarettes per day, current smokers who smoke 15-24 cigarettes per day, current smokers who smoke 25-34 cigarettes per day, and current smokers who smoke $\geq$35 cigarettes per day) and the categories for toenail nicotine levels using the same percent cut points as for the categories of reported smoking status. Although the toenail nicotine level was strongly associated with smoking level (Spearman $r = 0.63$), there was no complete concordance. For example, only 29% of women in category 3 of toenail nicotine levels came from category 3 of reported smoking, whereas more than 20% in that toenail nicotine levels category 3 came from category 1 of reported exposure (never smoking). Similarly, only 26% of women with the highest toenail nicotine levels came from women with the highest reported number of cigarettes smoked.

Table 1. Characteristics of the study population and the distribution of reported smoking and toenail biomarker

	n (median)	5-95 percentile range
Age (y)	2,485 (56.0)	42-62
Physical activity (h/wk)	2,027 (2.0)	1-4
Alcohol intake* (g/d)	2,217 (1.8)	0.0-32.8
Body mass index	2,375 (23.9)	19.4-34.3
Toenail nicotine for all participants (ng/mg)	2,485 (0.21)	0.06-4.06
Toenail nicotine for never smokers with no SHS exposure (ng/mg)	157 (0.10)	0.05-0.41
Toenail nicotine for never smokers with SHS exposure (ng/mg)	768 (0.14)	0.05-0.83
Toenail nicotine for current smokers (ng/mg)	771 (1.77)	0.24-6.34
	n	% of Population
Never smokers	988/2,485	40
Past smokers	726/2,485	29
Current smokers	771/2,485	31
SHS among never smokers	768/2,314	33
Occasionally		70
Frequently		30
Toenails infected with fungus	568/2,485	23
Nail polish used	122/2,485	4.9

*Using 1980 questionnaire 2 y before the toenail collection.

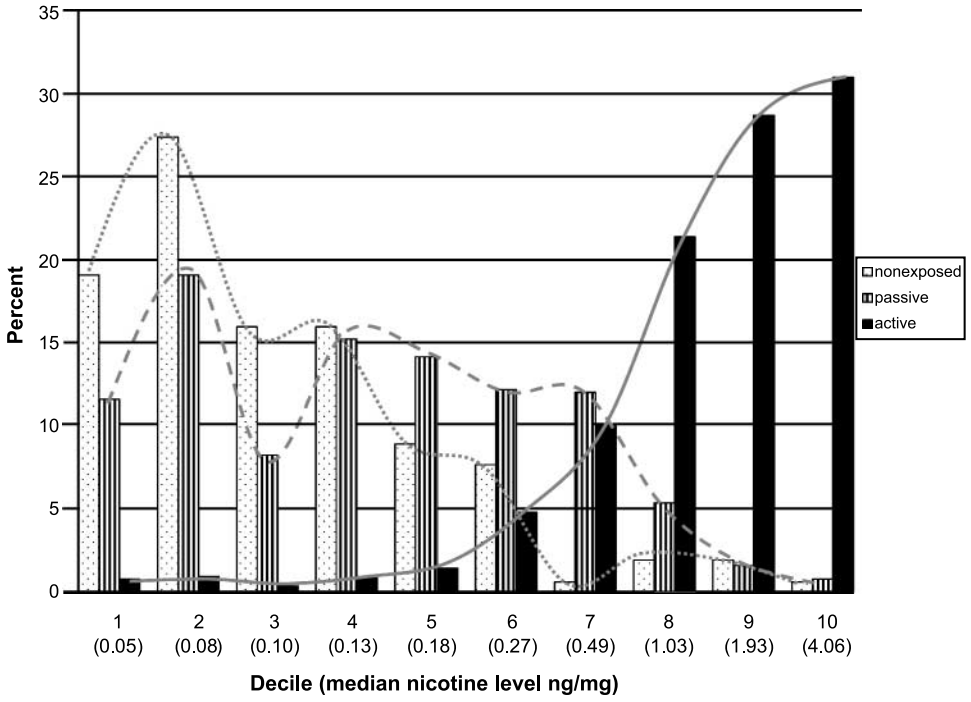

Figure 1. Percent distribution of reported smoking status according to toenail nicotine level deciles.

To examine the association between smoking and toenail nicotine level in a multiple regression model, we describe in Table 3 different models adjusted for age, where each model included a different reported categorical smoking variable (described in Materials and Methods) as a predictor of toenail nicotine levels. Based on the R^2 value, all the models were comparable in predicting toenail nicotine levels, except for the model for never smokers (model 5). The single smoking variable that best predicted the log-transformed nicotine levels was the number of cigarettes smoked. The reported smoking variable that least predicted nicotine levels was the frequency of SHS exposure among never smokers. Age consistently inversely and significantly predicted toenail nicotine levels in all the models. Reported SHS variable significantly predicted toenail nicotine levels even after including the number of cigarettes smoked in the model (see model 2 in Table 3). Reports of frequent SHS exposure were more strongly predictive of toenail nicotine levels than occasional SHS exposure (see models 4 and 5 in Table 3).

We also examined the value of toenail nicotine levels as an indicator of long-term smoking behavior by comparing toenail nicotine levels in 1982 with reported smoking at the first survey of NHS (as number of cigarettes per day) in 1976 and there was a good

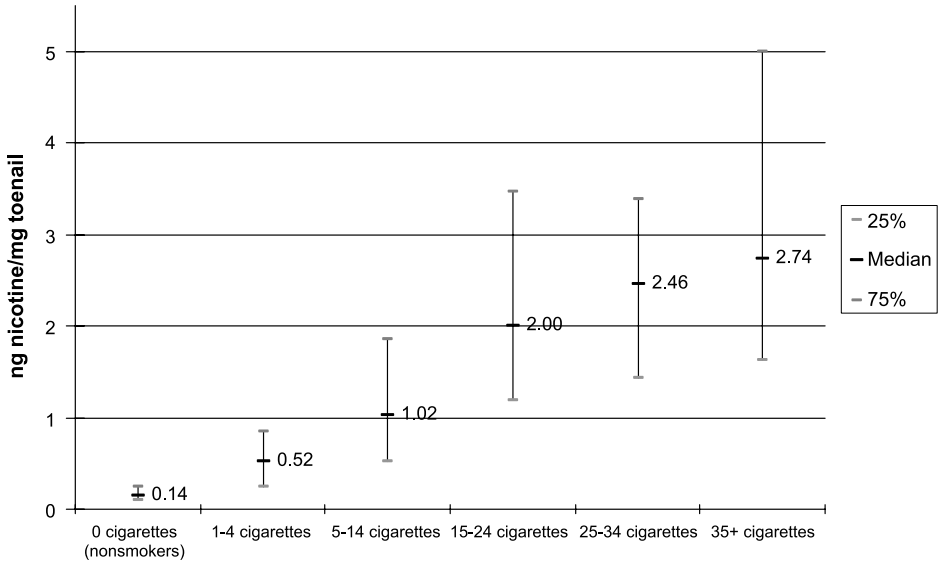

Figure 2. Median and 25-75% percentiles of toenail nicotine levels according to the number of cigarettes.

Table 2. Frequency of distribution of reported smoking status according to toenail nicotine level categories

	Toenail nicotine categories based on reported smoking status, % distribution in the population					
	1	2	3	4	5	6
	(0.01-0.14), 38.2	(0.15-0.62), 30.4	(0.63-1.23), 9.1	(1.24-2.83), 12.4	(2.84-4.80), 6.2	(4.81-32.7), 3.7
Smoking status						
1 Never smokers (39.3%)	57.7 (549)	48.2 (364)	20.4 (46)	7.5 (23)	2.0 (3)	3.3 (3)
2 Past smokers (29.4%)	40.1 (381)	37.6 (284)	19.0 (43)	4.6 (14)	1.3 (2)	2.2 (2)
3 Current: 1-14 cigarettes per day (9.1%)	1.1 (10)	9.13 (69)	28.8 (65)	18.8 (58)	11.8 (18)	4.4 (4)
4 Current: 15-24 cigarettes per day (12.4%)	0.7 (7)	3.7 (28)	19.5 (44)	37.0 (114)	50.3 (77)	39.6 (36)
5 Current: 25-34 cigarettes per day (6.2%)	0.4 (4)	0.8 (6)	7.5 (17)	21.8 (67)	23.5 (36)	24.2 (22)
6 Current: ≥35 cigarettes per day (3.6%)	0 (0)	0.7 (5)	4.9 (11)	10.4 (32)	11.1 (17)	26.4 (24)
	100	100	100	100	100	100

correlation (Spearman r = 0.63). The Spearman r for smoking categories in 1982 and toenail nicotine levels from the same year was stronger but comparable (r = 0.71). However, the correlation declined after 12 years of follow-up in 1994 (r = 0.48) over which time there was a decline in the percent of smokers.

Discussion

The overall burden of tobacco smoke among passive and active smokers is captured well by toenail nicotine levels, which can thus provide an objective measure of smoking exposure. There was a substantial overlap in

Table 3. Multivariate regression models for prediction of log-transformed toenail nicotine levels (ng nicotine/mg toenail) according to different reported smoking variables (n = 2,485)

Model number and tobacco exposure	Tobacco category	n	Estimate	SE	P	R^2
Model 1						
Smoke exposure	No exposure (reference group)	157	—	—	—	0.57
	SHS exposure among never smokers	768	0.35	0.08	<0.0001	
	Current active smoking	771	2.59	0.08	<0.0001	
Model 2						
Smoking status	Never smokers (reference group)	988	—	—	—	0.57
	Past smokers	726	0.07	0.05	0.15	
	Current smokers	771	2.29	0.04	<0.0001	
SHS exposure	No (reference group)	315	—	—	—	
	Yes	1,999	0.29	0.06	<0.0001	
Model 3						
No. cigarettes smoked per day	0 (reference group)	1,714	—	—	—	0.59
	1-4	44	1.08	0.14	<0.0001	
	5-14	180	1.80	0.07	<0.0001	
	15-24	306	2.43	0.06	<0.0001	
	25-34	152	2.59	0.08	<0.0001	
	≥35	89	2.80	0.10	<0.0001	
Model 4						
Frequency of SHS among everyone exposure	No exposure (reference group)	315	—	—	—	0.61
	Occasional	1,227	0.21	0.06	0.002	
	Frequent	772	0.46	0.06	<0.0001	
No. cigarettes smoked per day	0 (reference group)	1,714	—	—	—	
	1-4	44	1.03	0.14	<0.0001	
	5-14	180	1.76	0.07	<0.0001	
	15-24	306	2.36	0.06	<0.0001	
	25-34	152	2.50	0.08	<0.0001	
	≥35	89	2.70	0.10	<0.0001	
Model 5 (among never smokers with SHS frequency data; n = 925)						
Frequency of SHS exposure	No exposure (reference group)	157	—	—	—	0.04
	Occasional	538	0.26	0.08	0.001	
	Frequent	230	0.58	0.09	<0.0001	
Model 6						
Composite smoking status	Never smokers (reference group)	988	—	—	—	0.59
	Past smokers	726	0.07	0.04	0.10	
	Current smokers (1-14 cigarettes per day)	224	1.69	0.07	<0.0001	
	Current smokers (15-24 cigarettes per day)	306	2.46	0.06	<0.0001	
	Current smokers (25-34 cigarettes per day)	152	2.62	0.08	<0.0001	
	Current smokers (≥35 cigarettes per day)	89	2.83	0.10	<0.0001	

NOTE: Age (y) was significant in all the models. The following were not significant in any model and were excluded from the final models: nail fungus (yes, no), nail polish (yes, no), body mass index (<21, 21-22.9, 23-24.9, 25-28.9, ≥29), physical activity (<1, 1-1.9, 2-3.9, 4-6.9, ≥7 h/wk of moderate to vigorous activity), and alcohol intake (0, 0.1-4.9, 5-14.9, ≥15 g/d).
There were 169 participants (including 63 never smokers) that did not have information about SHS and were excluded from relevant models.

nicotine levels among groups defined by smoking history, especially among nonsmokers who are exposed to SHS and nonsmokers reporting no SHS exposure. The results thus suggest that toenail nicotine levels provide additional information on exposure that may not be fully captured by even a detailed history of active and passive smoking.

The slow growth rates of toenails overcome the day-to-day variability of exposure, especially to SHS, and provide a more stable estimate of average exposure. The use of toenails is a novel way to measure more objectively active and passive exposure to tobacco smoke, which has in the past been largely measured by self-reported questionnaires or short-lived biomarkers vulnerable to daily variability. Toenail nicotine levels may provide an alternative or complementary assessment of associations with health risk (compared with questionnaires alone) because it is measuring a related but different aspect of the same exposure.

Theoretically, we would have expected that active smokers would be limited to the highest deciles of nicotine levels, but our data show that some active smokers had nicotine levels similar to nonsmokers. This small number of smokers could be occasional smokers who smoke only a few cigarettes per week. For the nonsmokers who reported no exposure to SHS, many of them had toenail nicotine levels that were similar to those exposed to SHS, and a few were as high as some active smokers. This indicates possible unawareness of exposure to SHS or by being in places where recent smoking took place (22). This could also be explained by lower precision in the measurement of toenail nicotine level at low levels of exposure. The % coefficient of variation for levels below 0.15 ng/mg (which is where most of the nonsmokers fall) was 13.8% compared with a % coefficient of variation of 8.3% for the levels equal or above 0.15 ng/mg. As expected, women who reported never being smokers but were exposed to SHS were overlapping with both women nonexposed to SHS and active smokers. The method of analyses can be further refined to be more sensitive, but this would require larger amount of toenail clippings per person.

As noted earlier, reporting of SHS is problematic because of the many variables that influence the individual exposure (6). This might explain why some studies fail to find low-level risks between SHS and multifactorial disease. The reference groups in such studies are those who report not being exposed to SHS, whereas our data indicate the nicotine levels of both groups widely overlap, which would lead to attenuation of associations or inability to detect true association. Relying on toenail biomarkers to produce cutoff points for differentiating those exposed to SHS and those not exposed is also problematic because there are no population-based measures of these biomarkers. Attempts by several investigators to use cutoff points for a more widely used biomarker of SHS, such as cotinine, have failed to reach consistent values (23-26).

Titration of nicotine and smoking topography could explain the overlap in nicotine levels among those who smoked more than 15 cigarettes per day. Several studies indicate that smokers tend to titrate their nicotine levels regardless of the type or number of cigarettes they actually smoke (27). Other biomarkers also failed to differentiate between smokers who reported smoking different numbers of cigarettes (12, 28). The discrepancy between the categories of reported exposure and the respective categories of toenail nicotine levels using the same percent distribution also suggest misclassification or dissimilarity in how the two measures assess the exposure, although the correlation between the two measures is relatively high (r = 0.63), indicating good validity. Some of the discordance might also be due to variability in nicotine incorporation into nails and laboratory measurement error. We cannot distinguish the relative contribution of errors in the two methods to the discordance unless we compare them with a third independent method. In addition, in this study, we were not able to assess gender or ethnicity differences in relation to nail nicotine levels because the NHS included only women who are predominantly White. Age was inversely related to toenail nicotine levels, which is consistent with findings for other compounds measured in toenails such as selenium (29), arsenic (30), and calcium (31). This could be attributed to age-related change in the rate of incorporation of nicotine and these compounds into toenails (29) or lower efficiency of toenail circulation with age (31). Therefore, proper adjustment for age in future toenail nicotine studies is important.

In conclusion, this large study of toenail nicotine levels as biomarkers of exposure to tobacco smoke showed that toenail nicotine levels have high validity as a measure of both SHS exposure and active smoking. Thus, this biomarker can be used as an objective measure of exposure that may be particularly useful where bias is possible, such as in a case-control study or in monitoring compliance in smoking intervention trials. The considerable overlap in nicotine levels among persons reporting no exposure to SHS and those with reported exposure, as well as among categories of active smoking, suggest that toenail nicotine levels may be providing information on the overall burden of tobacco smoke exposure that is not captured by reported smoking history alone. The biomarker offers the opportunity to carry out new studies of tobacco use and its relation to health risks.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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