

Smoking, Secondhand Smoke, and Cotinine Levels in a Subset of EPIC Cohort

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Abstract

Background: Several countries are discussing new legislation regarding the ban on smoking in public places, based on the growing evidence of the hazards of secondhand smoke (SHS) exposure. The objective of the present study is to quantitatively assess the relationship between smoking, SHS, and serum cotinine levels in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort.

Methods: From a study on lung cancer in the EPIC cohort, questionnaire information on smoking was collected at enrolment, and cotinine was measured in serum. Three statistical models were applied by using samples available in a cross-section design: (i) cotinine levels by categories combining smoking and SHS ($n = 859$); (ii) the effect of hours of passive smoking exposure in nonsmokers only ($n = 107$); (iii) the effect of the number of cigarettes consumed per day in current smokers only ($n = 832$). All models were adjusted for country, sex, age, and body mass index.

Results: Among nonsmokers, passive smokers presented significant differences in cotinine compared with nonexposed, with a marked (but not significant) difference among former-smokers. A one hour per day increment of SHS gave rise to a significant 2.58 nmol/L (0.45 ng/mL) increase in mean serum cotinine ($P < 0.001$). In current smokers, a one cigarette per day increment gave rise to a significant 22.44 nmol/L (3.95 ng/mL) increase in cotinine mean ($P < 0.001$).

Conclusions: There is clear evidence that not only tobacco smoking but also involuntary exposure increases cotinine levels.

Impact: This study strengthens the evidence for the benefits of a smoking ban in public places. *Cancer Epidemiol Biomarkers Prev*; 20(5); 869–75. ©2011 AACR.

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Introduction

The carcinogenic effect of tobacco smoke was established when Doll and Hill conducted the first large epidemiological study in 1950 (1). Today, smoking is recognized as a major contributing factor in a vast variety of conditions including cancers, vascular, and respiratory diseases (2–4), not only to the individual who smokes but to others exposed to secondhand smoke (SHS; refs. 5, 6). Consequently in 2002, the International Agency for Research on Cancer (IARC) Working Group classified SHS as a group I human carcinogen (7, 8). Currently, several countries are discussing new legislation on the ban of smoking in public places; however, the World Health Organization (WHO) reported that only 17 countries had smoke-free policies that provide universal and effective protection from SHS in 2008 (9).

Despite the fact that self-administered questionnaires are frequently limited in terms of reliability and validity, they are routinely used to investigate smoking habits. Gorber and colleagues (10) have suggested that self-reported estimates may underestimate true smoking prevalence, with higher discrepancies in populations in which smoking is socially undesirable. Therefore, owing to the importance of smoking status and exposure to SHS for epidemiological research, biomarkers have been developed. Cotinine is the main metabolite of nicotine and a reliable biomarker of recent nicotine intake (11, 12). Although some foods contain small amounts of nicotine, cotinine level is considered as a reliable indicator of recent exposure to SHS (13).

Cotinine has a half-life of about 15 to 40 hours, reflecting both active and passive smoking during previous days (14). This biomarker has been linked to several modulating factors, including gender, body mass index (BMI), race, number of cigarettes, types of cigarette, filter, and the way the product is smoked (15, 16). Pickett and colleagues (17) have compared serum cotinine levels in nonsmokers living under extensive, limited, and no coverage smoke-free laws in different locations in the United States. To ensure that the sample included only nonsmokers, they used information from self-administered questionnaires and only included subjects who had serum cotinine levels lower or equal to 56.8 nmol/L (10 ng/mL). Subjects who declared passive smoke at home were also excluded from the sample. The results showed that among nonsmoking adults living in counties with extensive smoke-free law coverage, 12.5% were exposed to SHS, compared with 35.1% with limited coverage and 45.9% with no coverage.

In a meta-analysis of studies of passive smoking and lung cancer (18) among never-smoking females exposed to spousal smoking, the pooled relative risks were 1.22 in

5 cohort studies and 1.18 and 1.33, in 23 population-based case-control studies and 22 non-population-based case-control studies, respectively. The authors highlighted the fact that although the excess risk from SHS exposure is small, the high prevalence of this exposure makes it an important risk factor for lung cancer among nonsmokers.

Vineis and colleagues (8, 19) previously explored the carcinogenic effects of SHS in the European Prospective Investigation into Cancer and Nutrition (EPIC) study and observed that nonsmokers (never- and former-smokers) subjected to environmental smoke are at greater risk of lung cancer than subjects with no exposure. They estimated from the EPIC cohort that among never- and former-smokers, the SHS attributable proportion for lung cancer was 16%.

The objective of the present study is to quantitatively assess the relationship between smoking status, SHS, and cotinine levels in serum, by using a nested subcohort within the EPIC cohort.

Methods

The EPIC cohort

The EPIC is an ongoing multicentre European cohort study which recruited more than 520,000 healthy volunteers from 23 centres in 10 countries (Sweden, Denmark, Norway, the Netherlands, the United Kingdom, France, Germany, Spain, Italy, and Greece) between 1993 and 1998. The study includes men and women aged mainly between 35 and 74 at recruitment. Dietary and nondietary (lifestyle, anthropometric) information was collected through 2 questionnaires at enrolment. Further details of this study have been published previously elsewhere (20).

The current study utilizes data from a nested case-control within the EPIC cohort, which includes 899 cases of lung cancer and 1770 controls, matched by country, gender, date of blood collection, and date of birth (21). Subjects with missing information for smoking status, serum cotinine level, sex, age, or BMI were excluded from the sample.

Consent was obtained from all participants for the use of their blood samples for future analyses. The study was approved by local ethics committees in the participating countries and the ethical review board of the IARC.

Self-reported smoking

Information on SHS at home or at the workplace was collected in 13 out of 23 EPIC centers (6 in France, 5 in Italy, 1 in the Netherlands, and 1 in Germany), including home and workplace exposures, by using questions such as: "Does someone regularly smoke at home?" and "At work, are there people smoking in your presence?". The

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Table 1. Descriptive statistics for cotinine^a by smoking and SHS categories (*n* = 859), controlling for country, age, sex, and BMI (model 1)

Category	<i>n</i> = 859	Adjusted cotinine mean (nmol/L)	Standard error	95% confidence limits
NeSNP	125	2.98	0.39	2.21–3.74
NeSP	129	6.05	0.56	4.95–7.15
FSNP	114	3.81	0.67	2.51–5.12
FSP	37	9.66	2.16	5.42–13.90
CS	454	1286.83	53.76	1181.45–1392.20

^aTo convert nmol/L to ng/mL divide cotinine mean by the factor 5.68.

magnitude of SHS exposure was assessed by using questions on the number of hours per day of passive smoking each participant was exposed to (information for France and Italy) and the number of cigarettes per day smoked at home and at the workplace (information for France, Italy, Spain, the Netherlands, Greece, and Sweden).

Laboratory analyses

Participants were also asked to provide a blood sample at recruitment, following a standardized protocol.

Serum cotinine measurements were available for 2,699 participants within the nested case-control study design. Laboratory analysis was carried out at Bevitall AS (Bergen, Norway). The concentration of cotinine was determined by mass spectrometry-based methods (liquid chromatography coupled to tandem mass spectrometry and gas chromatography coupled to tandem mass spectrometry) with sensitivity of 1 nmol/L (0.18 ng/mL) and limit of quantification of 2 nmol/L (0.35 ng/mL; ref. 22). Nondetected serum cotinine levels were set at 0 nmol/L.

Statistical analysis

To evaluate the effect of smoking status as well as SHS on cotinine levels, subjects were classified according to the following criteria: never-smoker and no-passive exposure (NeSNP), never-smoker and passive exposure (NeSP), former-smoker and no-passive exposure (FSNP), former-smoker and passive exposure (FSP), and current smoker (CS); passive exposure could be at home or at the workplace.

Information on passive exposure (yes or no) was available only for the samples from France, Italy, the Netherlands, and Germany. As the distribution of serum cotinine was asymmetrical, for our analytical purposes we approximated it to a gamma distribution and transformed the data by adding 1 unit to all values of serum cotinine (nmol/L), to deal with 0 serum cotinine levels. This only affects the intercept (shifting the curve), not affecting the estimate of the slopes. Then, the association between smoking categories and cotinine levels was assessed by using a generalized linear model (GLM) with gamma distribution, adjusting for age, sex, BMI, and country (because nicotine contents may differ among

brands of cigarettes). It has been reported that age, sex, and BMI modify the relationship of number of cigarettes to cotinine (15, 16). BMI was considered as a confounder because subjects with a higher BMI tend to exhibit lower cotinine concentrations, possibly because of the distribution of cotinine into a bigger volume of blood (16). Females may take smaller and shorter puffs (23) and smoking behavior may differ accordingly to age. The sample size for this analysis was 859.

We built 2 further GLMs to evaluate: (i) the effect of hours of SHS per day on cotinine levels in nonsmokers (never and former). Information about hours of SHS per day was available only in the samples from France and Italy (*n* = 107). (ii) the effect of the average number of cigarettes currently smoked per day on serum cotinine in smokers (*n* = 832, information available from France, Italy, Spain, the United Kingdom, the Netherlands, Greece, Germany, and Sweden). In this latter model a better fit was achieved after adjusting for confounders considering the normal distribution.

All statistical analyses were carried out by using SAS 9.2 for Windows (SAS Institute).

Results

Among 3,818 subjects, 2,669 had information on cotinine level and 859 on both cotinine and passive smoke exposure. Some subjects who had declared not being smokers presented very high levels of cotinine. Usually people with cotinine levels higher than 80 nmol/L (14.08 ng/mL) are considered as active smokers (24). We excluded 9 self-declared nonsmokers with cotinine levels higher than 500 nmol/L (88.03 ng/mL; more than 2.65 SD away from the mean in nonsmokers), considered to be outliers. This exclusion affected participants from the following exposure categories (serum cotinine levels in nmol/L): 2 NeSNPs (893.23 and 1511.2), 5 FSNPs (1053.1, 2480.85, 1249.13, and 668.63), and 2 FSPs (555.59 and 523.78). We compared 5 categories of smoking and passive smoking as NeSNP, NeP, FSNP, FSP, and CS.

Tobacco exposure categories were found to be significant predictors (*P* < 0.01) of serum cotinine levels adjusting for country, sex, age, and BMI. Table 1 shows

Table 2. Mean comparison between pairs of smoking and SHS categories (model 1)^a

	Adjusted mean difference ^b (<i>P</i> value; 95% confidence limits)			
	NeSP	FSNP	FSP	CS
NeSNP	3.07 (<i>P</i> < 0.001; 1.92–4.22)	0.84 (<i>P</i> = 0.29; –0.71–2.39)	6.68 (<i>P</i> = 0.003; 2.20–11.16)	1283.85 (<i>P</i> < 0.001; 1178.47–1389.23)
NeSP		–2.24 (<i>P</i> = 0.02; –4.06–0.41)	3.61 (<i>P</i> = 0.12; –0.94–8.15)	1280.7 (<i>P</i> < 0.001; 1175.39–1386.16)
FSNP			5.85 (<i>P</i> = 0.01; 1.37–10.32)	1283.01 (<i>P</i> < 0.001; 1177.63–1388.39)
FSP				1277.16 (<i>P</i> < 0.001; 1171.70–1382.63)

^aGLM model controlling for country (*P* = 0.34), sex (*b* = 1.44, *P* = 0.04), age (*b* = –0.025, *P* = 0.41), and BMI (*b* = 0.19, *P* = 0.008), with Gamma distribution, intercept of 1,283.16 (1,176.64 – 1,388.67).

^bTo convert nmol/L to ng/mL divide cotinine mean by the factor 5.68.

serum cotinine means, whereas Table 2 shows the comparisons between pairs of exposure categories.

Among non-SHS exposed subjects, there was no significant difference in serum cotinine concentrations between never- and former-smokers (mean difference of 0.84 nmol/L or 0.15 ng/mL, *P* = 0.29), whereas the cotinine levels of current smokers were considerably higher than the 2 other categories of nonactive smoking; 1283.85 nmol/L or 226.03 ng/mL higher than never-smokers (*P* < 0.01) and 1283.01 nmol/L or 225.88 ng/mL higher (*P* < 0.01) than former-smokers.

Never-smoker and passive exposure had cotinine concentrations that were 3.07 nmol/L (0.54 ng/mL) higher than NeSNP. This was similar for former-smokers where FSP compared with FSNP presented cotinine concentrations 5.85 nmol/L (1.03 ng/mL) higher (*P* = 0.01). Among those who were passively exposed to cigarette smoke, there was a 3.61 nmol/L (0.64 ng/mL) difference in cotinine concentrations between never- and former-smokers that was not statistically significant (*P* = 0.12).

Furthermore, in this analysis the effect of country and age were nonsignificant (*P* = 0.34 and *P* = 0.41, respectively), but being male increased serum cotinine levels by

1.44 nmol/L (0.25 ng/mL; *P* = 0.04). The effect of BMI was also significant with an increase of 0.19 nmol/L (0.03 ng/mL) per unit increase in BMI (*P* = 0.01).

In the sample with information on the number of hours of passive smoking (*n* = 107, with 2 outliers deleted), an increase of 1 hour of passive smoking a day was associated with a 2.58 nmol/L (0.45 ng/mL; *P* < 0.01) higher mean serum cotinine concentration after controlling for country, age, sex, and BMI (Table 3). Country, sex, age, and BMI presented no effect (*P* = 0.86, 0.51, 0.88, and 0.30 respectively).

Among current smokers (*n* = 832), an increase of 1 cigarette per day was associated with a 22.44 nmol/L (3.95 ng/mL; *P* < 0.01) higher average serum cotinine level after controlling for country, age, sex, and BMI (Table 4). In this model there are differences of cotinine levels among countries (Table 4, *P* < 0.01). In addition, being male increases cotinine levels by 109.9 nmol/L (19.35 ng/mL) compared with females (*P* = 0.03), and each increase in BMI reduces cotinine levels by 31.88 nmol/L (5.61 ng/mL, *P* < 0.01).

As reported in Table 1, cotinine levels are much higher for smokers. It is possible to estimate the

Table 3. Generalized linear model for nonsmokers (never and former) exposed to SHS

Model 2 ^a (<i>n</i> = 107)		Estimate ^b	Standard error	Wald 95% confidence limits	<i>P</i>
Nonsmoker	Hours per day of passive smoking	2.58	0.60	1.41–3.75	<0.01
Country (France/Italy)	(Italy as baseline)	0.18	1.00	–1.79–2.15	0.86
Sex	Male/female	0.51	0.83	–1.12–2.14	0.54
Age	Years	–0.01	0.05	–0.12–0.10	0.88
BMI	kg/m ²	–0.15	0.14	–0.42–0.13	0.30

^aGLM with gamma distribution, intercept of 6.04 (–2.64–14.73).

^bPer unit increase, to convert nmol/L to ng/mL, divide cotinine mean by the factor 5.68.

Table 4. GLM for cotinine^a levels among smokers

Model 3 ^b (n = 832)		Estimate ^c	Standard error	Wald 95% confidence limits	P
Smokers	Per cigarettes per day	22.44	1.81	18.90–25.98	<0.01
Country (Greece as baseline)	France/Greece	26.81	163.47	–293.58–347.20	0.87
	Italy/Greece	149.85	76.67	–0.43–300.12	0.05
	Spain/Greece	80.39	75.42	–67.43–228.20	0.29
	UK/Greece	158.98	85.99	–9.56–327.53	0.06
	NL/Greece	269.80	79.96	113.08–426.52	<0.01
	Germany/Greece	206.63	71.87	65.76–347.49	<0.01
Sex	Male/female	109.09	50.42	10.26–207.92	0.03
Age	Years	–2.22	2.70	–7.51–3.06	0.41
BMI	kg/m ²	–31.88	4.89	–41.48–22.29	<0.01

^aTo convert nmol/L to ng/mL divide cotinine mean by the factor 5.68.

^bGLM with normal distribution and intercept of 2,194.281,793.87–2,594.68).

^cPer unit increase.

expected level of cotinine for 1 person with defined characteristics regarding the covariates (Table 4). For example, 1 female subject from Greece, 40 years old, BMI of 22, who smokes 1 cigarette per day, has an expected value of 1426.40 nmol/L (251.1 ng/mL) for cotinine levels. If she smokes 2 cigarettes per day, her expected level will increase to 1448.83 nmol/L (255.1 ng/mL). For a male subject, these values would be 1535.49 nmol/L (270.3 ng/mL) and 1557.92 (274.3 ng/mL), respectively.

Discussion

In this study, we have applied 3 general linear models to investigate how components of smoking exposure are quantitatively related to serum cotinine levels (15, 16, 23). The first model showed that among nonsmokers without SHS exposure, cotinine levels did not differ between never- and former-smokers. Among those who were passively exposed, FSP had slightly higher cotinine concentrations on average when compared with NeSP, but the difference was not significant ($P = 0.12$). The sample size of the FSP group is quite small ($n = 37$) with average cotinine levels of 9.66 nmol/L (1.70 ng/mL) and a large standard error (2.16 nmol/L). The 95% confidence limits presents a lower limit of 5.95 nmol/L (1.05 ng/mL) which is quite lower than the upper limit 8.15 nmol/L (1.43 ng/mL) of the NeSP group. The number of passive smoking hours are similar between the 2 categories NeSP and FSP (means of 1.28 and 1.17 hours, respectively), but it should be noted that in the sample size for former-smokers with passive smoking hours, information is limited ($n = 6$ data not shown). In a recent paper, Piccardo and colleagues suggested that among smokers the exposure to their own indoor environmental smoke is not negligible, if smoking occurs in indoor environments (25). We did not compare cotinine levels among current smokers with and without passive exposure.

In our results, it is clear that among nonsmokers, passive exposure significantly increases serum cotinine levels (never-smokers: $P < 0.01$, and former-smokers: $P = 0.01$). In general, 1 extra hour of passive exposure is associated with a 2.58 nmol/L (0.45 ng/mL) increase in cotinine levels. A range of studies show that cotinine levels are positively related to the number of hours of passive tobacco exposure (26) or with the number of cigarettes actively smoked (10, 15, 16, 24), but none have compared never- and former-smokers. Whincup and colleagues (6) found an excess of risk of coronary heart disease for higher concentrations of serum cotinine among nonsmokers; this is strengthened by the increase in risk of dying from cardiovascular diseases from home SHS exposure observed by Gallo and colleagues (5).

Being exposed to SHS increases serum cotinine levels by 3.07 nmol/L (0.54 ng/mL) in never-smokers and 5.85 nmol/L (1.03 ng/mL) in former-smokers. Vineis and colleagues (8) suggested that former-smokers may be more susceptible to the effects of environmental tobacco smoke, having higher levels of cotinine when compared with never-smokers, after controlling for number of hours of passive smoking. The fact that cotinine levels were very similar between former- and never-smokers suggests that cotinine levels reduce upon cessation of smoking owing to the short half-life. However, we do not have data for cotinine levels by time since cessation, which is a limitation of the study.

Furthermore, it was found that cotinine levels were related to sex and BMI, in agreement with previous studies (15, 16). However, BMI is inversely associated with cotinine but this relation was not observed in nonsmokers. Females may metabolize nicotine differently from males because of hormonal factors, may take smaller and shorter puffs (as we adjusted for number of cigarettes smoked), men could have underreported actual use of tobacco or women could have over reported (23).

Our findings in current smokers concur with previous publications: cotinine levels increase when the mean number of cigarettes per day increases (15, 16, 24). As shown in Table 4, the magnitude of the rise was 22.44 nmol/L (3.95 ng/mL) for each increase in cigarette smoked per day.

Our results regarding the relationships between cotinine levels and categories of smoking status, hours of SHS per day, and number of cigarettes smoked per day show that the self-reported answers to the tobacco exposure questionnaire were accurate.

Strulovici-Barel and colleagues (27) evaluated bronchoscopy and genome-wide gene expression of 121 healthy volunteers from New York City. The group was separated into nonsmokers, active smokers, and individuals exposed to low levels of tobacco smoking (environmental tobacco exposure and/or occasional smoking). The results showed that the small airway epithelium is very sensitive to low-level tobacco smoke exposure (nicotine and cotinine levels were considered for this classification). They conclude that "the changes in gene expression are likely the earliest biological abnormalities in the small airway epithelium that lead to clinically detectable lung disease in some individuals." Hence it is possible that

high levels of SHS exposure (by using cotinine concentrations as proxy) may be relevant for lung cancer aetiology in nonsmokers.

Conclusions

We conclude that in accordance with previous studies, active tobacco smoking is associated with increases in serum cotinine levels in individuals. Furthermore, we find that SHS also increases serum cotinine levels: this effect may be stronger in former-smokers compared with never-smokers (not significant in this study with only 37 FSP subjects). We strongly support the proposed smoking ban which will provide protection to never-smokers and former-formers by reducing SHS exposure.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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