Serum Cotinine and Silent Myocardial Infarction in Individuals Free from Cardiovascular Disease

Ryan Brunetti, MD[a], Harry Hicklin, MD[a], Justin Rackley, MD[a], Muhammad Imtiaz Ahmad, MBBS, MS[b,*], Yabing Li, MD[c], and Elsayed Z. Soliman, MD, MSc, MS[c,d]

Serum cotinine is a sensitive and specific marker of tobacco exposure, including second-hand smoke exposure. We sought to explore the association of tobacco exposure determined by serum cotinine with electrocardiographic silent myocardial infarction (SMI). A total of 7,006 participants (59.0 ± 13.3 years; 52.6% women, 49.7% non-Hispanic whites) without cardiovascular disease from the Third National Health and Nutrition Examination Survey (NHANES III) were included in this analysis. SMI was defined as electrocardiographic evidence of MI in the absence of a history of MI. Multivariable logistic regression analysis was used to examine the association between SMI and serum cotinine tertiles. SMI was detected in 114 (1.63%) of the participants. The prevalence of SMI was higher among those with higher levels of serum cotinine (SMI prevalence was 1.25%, 1.49%, and 2.14% across serum cotinine lower [0.03 to 0.12 ng/ml], middle [0.12 to 1.39 ng/ml], and higher [1.40 to 1890 ng/ml] tertiles, respectively). In a model adjusted for potential confounders, participants within the highest serum cotinine tertile had significantly greater odds of SMI (odds ratio [95% confidence interval]: 2.51 [1.55 to 4.08]) compared with those with serum cotinine levels in the first tertile. Each 10 ng/ml increase in serum cotinine levels was associated with a 2% ($p < 0.0001$) increase in the prevalence of SMI. This association was stronger in white than nonwhite participants (interaction p value = 0.05). In conclusion, elevated serum cotinine levels are associated with SMI. These findings further highlight the risk associated with passive and active smoking on cardiovascular health and underscore the potential utility of serum cotinine in identifying those at risk. **(Am J Cardiol 2019;124:666−670)**

The association between tobacco exposure and cardiovascular disease (CVD) is well established; exposure to tobacco causes nearly 500,000 premature deaths in the United States annually, with approximately 40,000 of these attributed to second-hand smoke (SHS) exposure.[1] Tobacco use/exposure is usually assessed using self-report questionnaires. However, this method is prone to bias as participants may be unwilling to admit a health or social behavior perceived to be undesirable.[2,3] Due to challenges of accurate estimation of tobacco exposure in self-reported never smokers as well as in SHS,[2,4] a sensitive and specific biomarker of tobacco exposure such as serum cotinine has been proved very useful in estimating the risks associated with tobacco exposure in these groups.[5,6] Although smoking is an established risk factor for atherosclerosis and myocardial infarction,[1,7] its association with electrocardiographic silent myocardial infarction (SMI) using serum cotinine has not been explored previously. SMI accounts for almost half of total myocardial infarction and has been associated with increased risk of reinfarction, heart failure (HF), CVD, and all-cause mortality.[8−10] Early detection of SMI in high-risk groups with prevalent tobacco exposure may offer an opportunity for lifestyle changes to reduce the risks of future poor outcomes. Therefore, we proposed to examine the association of serum cotinine with SMI among participants free of clinical CVD using data from the Third National Health and Nutrition Examination Survey.

Methods

NHANES is a periodic survey of a representative sample of the civilian noninstitutionalized US population that provides estimates of disease prevalence and overall health status in the US population.[11] At the time of study enrollment, all participants provided written informed consent. Data from participants were collected from 1988 to 1994 through an in-home interview process with a subsequent visit to a mobile examination center.

Only participants who underwent electrocardiogram (ECG) recording as part of the NHANES III were included in the analysis. We excluded participants with a history of coronary heart disease, HF, or stroke, or those with missing cotinine level data. Of the 8,561 NHANES III participants, 7,006 participants were included in the final analysis.

Age, gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, and other), smoking status (never, current, and former), history of gout, and leisure time physical activity (number of times engaged in

[a]Department of Internal Medicine, Wake Forest School of Medicine, Winston Salem, North Carolina; [b]Department of Internal Medicine, Section on Hospital Medicine, Wake Forest School of Medicine, Winston Salem, North Carolina; [c]Department of Epidemiology and Prevention, Epidemiological Cardiology Research Center (EPICARE), Wake Forest School of Medicine, Winston Salem, North Carolina; and [d]Department of Internal Medicine, Section on Cardiology, Wake Forest School of Medicine, Winston Salem, North Carolina. Manuscript received March 24, 2019; revised manuscript received and accepted May 21, 2019.

Funding: None.

See page 669 for disclosure information.

*Corresponding author: Tel: (336)716-3674; fax: (336)716-0030.

E-mail address: muahmad@wakehealth.edu (M.I. Ahmad).

0002-9149/
https://doi.org/10.1016/j.amjcard.2019.05.064

physical activity in the past month) were self-reported. Diabetes was defined as a fasting plasma glucose level of ≥126 mg/dl or the use of glucose-lowering medications. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of antihypertensive medications. Obesity was defined as a body mass index of ≥30 kg/m^2. Hyperlipidemia was defined as serum cholesterol ≥200 mg/dl or serum triglycerides ≥150 mg/dl of use of lipid-lowering medications. Participants provided blood samples during visits to mobile examination centers to obtain baseline laboratory values. These included serum cotinine, total cholesterol, serum triglycerides, plasma glucose, and so on.

Biochemical determination of tobacco exposure was performed by measuring serum cotinine level in blood samples obtained at medical examination center. Serum cotinine was measured by an isotope dilution-high-performance liquid chromatography atmospheric pressure chemical ionization-tandem mass spectrometry.[12] Usual cut-off value range of 10 to 20 ng/ml is used to define smoker versus nonsmoker status.[13]

The 12-lead ECGs at rest were obtained by trained technicians with a Marquette MAC 12 system (Marquette Medical Systems, Milwaukee, Wisconsin) during the mobile examination visits. ECG analysis occurred through both a computerized automated process in a centralized core laboratory after visual inspection by trained technicians. Participants were determined to have an SMI if they reported no history of MI but had evidence of previous MI on ECG. ECG diagnosis of previous MI was defined using the Minnesota code (MC) as the presence of a major Q-wave abnormality (MC 1-1-X or 1-2-X) or minor Q/QS waves with major ST-T wave abnormalities (MC 1-3-X with 4-1-X, or 4-2, or 5-1, or 5-2).[14]

Baseline characteristics were compared across serum cotinine tertiles. Continuous variables were reported as mean ± standard deviation, whereas categorical variables were reported as frequency and percentage. Analysis of variance was used to compare the continuous variables, whereas chi-square test was used to compare the categorical variables.

A multivariable logistic regression analysis was used to compute odds ratios (ORs) and 95% confidence intervals (CI) of SMI across serum cotinine tertiles. We also calculated ORs and 95% CI for SMI prevalence per 10 ng/ml increase in serum cotinine level. In both approaches, model 1 was adjusted for age, gender, and race, whereas model 2 was adjusted for model 1 plus hypertension, obesity, diabetes, hyperlipidemia, and physical activity.

To evaluate for consistency throughout subgroups, we also examined the association between serum cotinine tertile and SMI stratified by race (white vs nonwhite), gender (men vs women), age (<60 years vs ≥60 years), obesity (obese vs nonobese), smoking status (never vs ever smoker), blood pressure (normotensive vs hypertensive), and diabetes (nondiabetics vs diabetics). We tested for interaction using models adjusted for variables similar to those included in model 2 with the addition of interaction term between main effect variable and subgroup.

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc. Cary, North Carolina) and p values were considered significant if <0.05.

Results

A total of 7,006 participants (59.0 ± 13.3 years; 52.6% women, 49.7% non-Hispanic whites) were included in the final analysis. Table 1 shows the baseline characteristics of the study participants stratified by serum cotinine tertiles. Participants in the third cotinine tertile were more likely to be young, male, nonwhite, and current smokers. They were less likely to be obese, had low physical activity levels, had lower cholesterol levels, lower systolic blood pressure, and less likely to have diabetes. The prevalence of SMI was 1.25%, 1.49%, and 2.14% in the first, second, and third serum cotinine tertiles, respectively.

In a model adjusted for demographics and potential confounders, participants with highest serum cotinine tertile had 151% increased prevalence of SMI (OR [95% CI]: 2.51 [1.55 to 4.08]) compared with those with the first tertile of serum cotinine levels (Table 2). Each 10 ng/ml increase in serum cotinine level was associated with a 2% increase in the prevalence of SMI (Table 2).

The association between serum cotinine tertiles and SMI was consistent across subgroups stratified by age, gender, obesity, smoking status, hypertension, and diabetes status. However, effect modification was evident with regard to race with white participants having significantly greater odds of SMI compared with nonwhite participants (interaction p value = 0.05; Table 3).

Discussion

In this analysis from a large racially diverse community-based sample of participants free of clinical CVD, we observed a strong association of serum cotinine with prevalent SMI. This association was stronger among white participants compared with nonwhites. These findings add to more concerns about the negative impact of tobacco exposure.

Cotinine is one of the primary products of nicotine breakdown and has a relatively long half-life of 18 to 20 hours compared with nicotine's half-life of 2 to 3 hours.[15] One distinct advantage of serum cotinine over simply relying on reported smoking history is the ability to ascertain the degree of SHS exposure. One study of the US workforce found that self-reported denial of any home or workplace SHS exposure in nonsmokers was only 28% accurate.[4] Studies examining serum cotinine levels in passive smokers found that a cotinine level of 2.8 to 14 ng/ml is associated with nearly the same risk of coronary artery disease as that in smokers consuming up to 9 cigarettes daily.[16] Although determining the degree of SHS is a challenge, the risks of SHS certainly cannot be disregarded. Therefore, an accurate means of assessing SHS is instrumental in advancing efforts to combat it and its effects. Of all potential biomarkers for tobacco exposure, serum cotinine is generally considered the gold standard for measuring tobacco exposure, including SHS.[17] In our study, we observed an association of serum cotinine among self-reported never smokers in subgroups; a finding that further supports the potential risks associated with SHS/exposure detected by serum cotinine.

The prognostic significance of SMI is well-established. The prognosis of SMI is similar or worse than clinically recognized MI.[10] SMI has been associated with increased risk

Table 1
Baseline characteristics of 7,006 study participants from NHANES-III

Characteristics	Serum cotinine tertiles			p value†
Mean ± SD or n (%)	1st (n = 2,329)	2nd (n = 2,342)	3rd (n = 2,335)	
Serum cotinine (ng/ml)	0.06±0.02	0.40±0.30	211.6±184.2	<.0001
*Serum cotinine (ng/ml)	0.03-0.12	0.12-1.39	1.40-1890	
Age (years)	62.5±13.8	58.2±13.1	56.4±12.4	<.0001
Men	865 (37.1%)	1074 (45.8%)	1382 (59.1%)	<.0001
Race				<.0001
Non-Hispanic whites	1278 (54.8%)	1161 (49.5%)	1043 (44.6%)	
Non-Hispanic blacks	269 (11.5%)	557 (23.7%)	769 (32.9%)	
Mexican Americans	678 (29.1%)	526 (22.4%)	445 (19.0%)	
Others	104 (4.4%)	98 (4.1%)	78 (3.3%)	
Systolic blood pressure (mm Hg)	134.0±20.3	131.9±19.3	130.8±19.2	<.0001
Diastolic blood pressure (mm Hg)	75.5±9.9	76.8±10.2	76.9±10.3	<.0001
Antihypertensive medications	540 (23.1%)	525 (22.4%)	392 (16.7%)	<.0001
Diabetes mellitus	309 (13.2%)	290 (12.3%)	252 (10.7%)	0.03
Anti-diabetics	184 (7.9%)	166 (7.0%)	133 (5.7%)	0.01
Total cholesterol (mg/dl)	219.8±43.8	219.2±43.4	213.6±43.6	<.0001
Serum triglycerides (mg/dl)	161.0±123.5	154.6±114.5	160.7±139.6	0.15
Anti-hyperlipidemic	85 (3.6%)	60 (2.5%)	54 (2.3%)	0.01
Smoker				
Never	1479 (63.5%)	1349 (57.6%)	353 (15.1%)	<.0001
Former	839 (36.0%)	970 (41.4%)	418 (17.9%)	<.0001
Current	11 (0.4%)	23 (0.98%)	1564 (66.9%)	<.0001
Obesity	660 (28.3%)	776 (33.1%)	534 (22.8%)	
‡Physical activity (*METs per week*)	12 (1.6-34.8)	10 (1.9-31.3)	7.5 (0-26.8)	<.0001
Silent myocardial infarction	29 (1.25%)	35 (1.49%)	50 (2.14%)	0.04

Obesity defined as body mass index ≥30 kg/m^2.
* Serum cotinine reported as a range.
† p Value calculated by ANOVA for continuous variables and chi-square test for categorical variables.
‡ METs per week reported as median and IQR.

of CHD, all-cause, CVD mortality, and incident HF.[9,18–20] In light of the findings of our study, a screening ECG may provide further risk assessment in participants with elevated serum cotinine levels. Considering the prognostic significance of SMI, early detection can offer the opportunity to employ preventive strategies such as lifestyle modifications, which can thereby reduce the risk of poor outcomes.

In our study, subjects with elevated serum cotinine seemed to carry less-traditional risk factors for CVD, finding observed previously too.[6,21] Interestingly, white participants were found to have significantly greater odds of SMI compared with nonwhite participants in this study. It has been demonstrated in multiple previous studies that cotinine levels in blacks are higher than those of whites at similar levels of tobacco exposure.[22–24] Potential mechanisms underlying such racial differences include the difference in pharmacokinetics related to cytochrome P450 activity (CYP2A6), different tobacco brands, mentholation, and smoking methods/habits.[24] Differences in serum cotinine levels despite the same level of tobacco exposure may explain racial differences in the association of serum cotinine with SMI in our study. However, the underlying biologic reasons for this heterogeneity are unclear and therefore, to better understand underlying mechanisms, future studies should consider racial differences when exploring the association of serum cotinine as a surrogate marker of tobacco exposure to study CVD outcomes.

Certain limitations of our study must be acknowledged when considering its results and implications. Due to the cross-sectional design of the study, temporality and residual confounding should be considered as a limitation of the study. A single measurement of serum cotinine may not be able to fully capture the chronic SHS exposure, another limitation of the study. We used ECG to diagnose SMI, and in comparison to the gold standard such as cardiac MRI, ECG has a relatively low sensitivity for detecting SMI.[25] Therefore, our reported prevalence of SMI may be underestimated, potentially causing an artificial attenuation of the positive relation between cotinine and SMI. Strengths of the study include a large sample size, comprised of a community-living, multiracial population, with better generalizability of

Table 2
ORs and 95% CI of cross-sectional association of serum cotinine with silent myocardial infarction

Serum cotinine	Model 1 OR (95% CI)	p value	Model 2 OR (95% CI)	p value
1st tertile	*Ref*	-	*Ref*	-
2nd tertile	1.52 (0.92-2.51)	0.10	1.50 (0.91-2.49)	0.11
3rd tertile	2.42 (1.49-3.91)	0.0003	2.51 (1.55-4.08)	0.0002
Per 10 ng/ml	1.02 (1.01-1.02)	<.0001	1.02 (1.01-1.03)	<.0001

CI = confidence interval; OR = odds ratio.
Model 1 adjusted for age, gender, and race.
Model 2 adjusted for model 1 plus hypertension, obesity, diabetes, hyperlipidemia, and physical activity.

Table 3
ORs and 95% CI of association of serum cotinine with silent myocardial infarction across subgroups

Subgroups	Serum cotinine tertiles	Events/participants	OR (95% CI)*	*Interaction p value*
White	2nd	22/1161 (1.9%)	2.60 (1.27-5.30)	0.05
	3rd	24/1043 (2.3%)	4.05 (1.94-8.45)	
Non-white	2nd	13/1181 (1.1%)	0.78 (0.37-1.64)	
	3rd	26/1292 (2.0%)	1.51 (0.79-2.88)	
Men	2nd	17/1074 (1.6%)	1.36 (0.64-2.90)	0.91
	3rd	33/1382 (2.4%)	2.44 (1.22-4.87)	
Women	2nd	18/1268 (1.4%)	1.60 (0.81-3.4)	
	3rd	17/953 (1.8%)	2.58 (1.28-5.22)	
<60 years	2nd	14/1366 (1.0%)	3.07 (0.87-10.8)	0.96
	3rd	16/1471 (1.1%)	3.39 (0.96-11.8)	
≥60 years	2nd	21/976 (2.1%)	1.10 (0.61-1.97)	
	3rd	34/864 (3.9%)	2.07 (1.22-3.54)	
Non-obesity	2nd	23/1566 (1.5%)	1.52 (0.82-2.81)	0.89
	3rd	39/1801 (2.1%)	2.64 (1.49-4.68)	
Obesity	2nd	12/776 (1.6%)	1.43 (0.59-3.48)	
	3rd	11/534 (2.0%)	2.22 (0.87-5.66)	
Never smoker	2nd	20/1349 (1.5%)	1.66 (0.85-3.23)	0.93
	3rd	10/353 (2.8%)	2.71 (1.19-6.15)	
Ever smoker	2nd	0/23 (0%)	1.30 (0.60-2.82)	
	3rd	30/1564 (1.9%)	2.24 (1.13-4.14)	
Normotensive	2nd	6/993 (0.6%)	3.67 (0.73-18.4)	0.48
	3rd	11/1050 (1.0%)	6.67 (1.43-31.0)	
Hypertensive	2nd	29/1349 (2.1%)	1.33 (0.77-2.28)	
	3rd	39/1285 (3.0%)	2.21 (1.30-3.73)	
Non-diabetics	2nd	28/2052 (1.4%)	1.83 (1.02-2.39)	0.21
	3rd	44/2083 (2.1%)	3.40 (1.94-5.95)	
Diabetics	2nd	7/290 (2.4%)	0.77 (0.27-2.18)	
	3rd	6/252 (2.4%)	0.79 (0.26-2.38)	

CI = confidence interval; ORs = odds ratio.
First tertile of serum cotinine (ref).
* Model adjusted for age, gender, race, hypertension, obesity, diabetes, hyperlipidemia, and physical activity.

this study to the US population. Cotinine has promising potential as a risk marker for CVD that is more objective than reported tobacco exposure, particularly regarding SHS exposure. With a more accurate measure of tobacco exposure, we may be able to further personalize and better implement CVD risk modification strategies.

Disclosures

The authors have no conflicts of interest to disclose.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2019.05.064.

1. National Center for Chronic Disease Prevention and Health Promotion Office on Smoking and Health. *The health consequences of smoking—50 years of progress: a report of the surgeon general*. Reports of the Surgeon General. Atlanta, GA: Centers for Disease Control and Prevention; 2014.
2. Patrick DL, Cheadle A, Thompson DC, Diehr P, Koepsell T, Kinne S. The validity of self-reported smoking: a review and meta-analysis. *Am J Public Health* 1994;84:1086–1093.
3. Caraballo RS, Giovino GA, Pechacek TF, Mowery PD. Factors associated with discrepancies between self-reports on cigarette smoking and measured serum cotinine levels among persons aged 17 years or older: Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* 2001;153:807–814.
4. Arheart KL, Lee DJ, Fleming LE, LeBlanc WG, Dietz NA, McCollister KE, Wilkinson JD, Lewis JE, Clark JD 3rd, Davila EP, Bandiera FC, Erard MJ. Accuracy of self-reported smoking and secondhand smoke exposure in the US workforce: the National Health and Nutrition Examination Surveys. *J Occup Environ Med* 2008;50:1414–1420.
5. Irfan A, Li Y, Bhatnagar A, Soliman EZ. Association between serum cotinine levels and electrocardiographic left atrial abnormality. *Ann Noninvasive Electrocardiol* 2019;24:e12586.
6. Ali M, Li Y, O'Neal WT, Soliman EZ. Tobacco exposure as determined by serum cotinine and subclinical myocardial injury in individuals free from cardiovascular disease. *Am J Cardiol* 2017;120:1114–1117.
7. Benowitz NL. Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. *Prog Cardiovasc Dis* 2003;46:91–111.
8. Pride YB, Piccirillo BJ, Gibson CM. Prevalence, consequences, and implications for clinical trials of unrecognized myocardial infarction. *Am J Cardiol* 2013;111:914–918.
9. Qureshi WT, Zhang ZM, Chang PP, Rosamond WD, Kitzman DW, Wagenknecht LE, Soliman EZ. Silent myocardial infarction and long-term risk of heart failure: the ARIC study. *J Am Coll Cardiol* 2018;71:1–8.
10. Zhang ZM, Rautaharju PM, Prineas RJ, Rodriguez CJ, Loehr L, Rosamond WD, Kitzman D, Couper D, Soliman EZ. Race and sex differences in the incidence and prognostic significance of silent myocardial infarction in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 2016;133:2141–2148.
11. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988-94. Series 1: programs and collection procedures. *Vital Health Stat 1* 1994:1–407.
12. Bernert JT, Turner WE, Akins J, Waldrep M. Development of a rapid and sensitive method for serum cotinine analysis as a marker of exposure to environmental tobacco smoke. *Clin Chem* 1994;40:1075.

13. Kim S. Overview of cotinine cutoff values for smoking status classification. *Int J Environ Res Public Health* 2016;13.
14. Prineas RJ, Crow RS, Blackburn H. *The Minnesota Code Manual of Electrocardiographic Findings: Standards and Procedures for Measurement and Classification*. Littleton, MA: J. Wright; 1982.
15. Benowitz NL, Jacob P 3rd. Daily intake of nicotine during cigarette smoking. *Clin Pharmacol Ther* 1984;35:499–504.
16. Whincup PH, Gilg JA, Emberson JR, Jarvis MJ, Feyerabend C, Bryant A, Walker M, Cook DG. Passive smoking and risk of coronary heart disease and stroke: prospective study with cotinine measurement. *BMJ* 2004;329:200–205.
17. Torres S, Merino C, Paton B, Correig X, Ramirez N. Biomarkers of exposure to secondhand and thirdhand tobacco smoke: recent advances and future perspectives. *Intl J Environ Res Public Health* 2018;15.
18. Kannel WB, Abbott RD. Incidence and prognosis of unrecognized myocardial infarction. An update on the Framingham study. *N Engl J Med* 1984;311:1144–1147.
19. Maradit-Kremers H, Crowson CS, Nicola PJ, Ballman KV, Roger VL, Jacobsen SJ, Gabriel SE. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum* 2005;52:402–411.
20. Ahmad MI, Dutta A, Anees MA, Soliman EZ. Interrelations between serum uric acid, silent myocardial infarction, and mortality in the general population. *Am J Cardiol* 2019;123:882–888.
21. Bakhru A, Erlinger TP. Smoking cessation and cardiovascular disease risk factors: results from the Third National Health and Nutrition Examination Survey. *PLoS Med* 2005;2:e160.
22. Wagenknecht LE, Cutter GR, Haley NJ, Sidney S, Manolio TA, Hughes GH, Jacobs DR. Racial differences in serum cotinine levels among smokers in the Coronary Artery Risk Development in (Young) Adults study. *Am J Public Health* 1990;80:1053–1056.
23. Caraballo RS, Giovino GA, Pechacek TF, Mowery PD, Richter PA, Strauss WJ, Sharp DJ, Eriksen MP, Pirkle JL, Maurer KR. Racial and ethnic differences in serum cotinine levels of cigarette smokers: Third National Health and Nutrition Examination Survey, 1988-1991. *JAMA* 1998;280:135–139.
24. Signorello LB, Cai Q, Tarone RE, McLaughlin JK, Blot WJ. Racial differences in serum cotinine levels of smokers. *Dis Markers* 2009;27:187–192.
25. Soliman EZ. Silent myocardial infarction and risk of heart failure: current evidence and gaps in knowledge. *Trends Cardiovasc Med* 2018;29:239–244.