Serum Cotinine Level and Incident Hearing Loss

A Case-Control Study

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Background: A growing body of literature suggests an association between cigarette smoking and hearing loss.

Objective: To assess the relation between levels of serum cotinine, a biomarker of exposure to tobacco smoke, and incident hearing loss.

Design: A cross-sectional, incident, case-control study of participants selected from a population-based cohort.

Setting: Testing was conducted at the Beaver Dam Community Hospital, Beaver Dam, Wis.

Participants: A total of 197 participants with incident hearing loss and 394 control participants, aged 53 to 75 years, selected from the 2800 participants of the 5-year follow-up examination of the population-based Epidemiology of Hearing Loss Study, 1998-2000.

Main Outcome Measure: Incident hearing loss. The incidence of hearing loss was defined as a pure-tone average of thresholds at 500, 1000, 2000, and 4000 Hz greater than 25-dB hearing level in either ear at follow-up among those without hearing loss at baseline.

Results: No significant associations were found between serum cotinine levels and incident hearing loss.

Conclusions: These results were not consistent with a previous report, which found cross-sectional associations between prevalent hearing loss and current smoking and environmental tobacco smoke exposure in the home. Longer-term longitudinal studies of smoking and/or serum cotinine levels and the subsequent development of hearing loss may help clarify these associations.

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HEARING LOSS IS ONE OF THE most common chronic conditions among older adults (≥65 years) in the United States, but its cause is not well understood. A recent population-based study of hearing loss in Beaver Dam, Wis, reported that 46% of adults aged 48 to 92 years had hearing loss. Among those in the Beaver Dam study without hearing loss at the baseline examination, 21% had developed hearing loss 5 years later. Because older adults are expected to compose an increasing proportion of the US population in years to come, the number of adults dealing with the impact of hearing loss on their everyday lives will likely increase as well.

While hearing loss is commonly associated with aging, some studies of rural African tribes have failed to find the usual decline in hearing sensitivity associated with age. In addition, several studies have indicated that cardiovascular disease or its risk factors may be associated with hearing loss.

Cigarette smoking, a known cardiovascular disease risk factor, may affect hearing through its effects on antioxidative mechanisms or on the vasculature supplying the auditory system. In a cross-sectional population-based study of 3571 older adults, an association between current smoking and hearing loss was reported, with current smokers 1.7 times as likely to have hearing loss as nonsmokers. In the same study, nonsmoking participants who lived with a smoker were almost twice as likely to have hearing loss as those who were not exposed to a household member who smoked, suggesting that environmental tobacco smoke exposure may also be associated with hearing loss. Several other studies have reported an association between smoking and hearing loss, while others have found no such association.

One of the limitations of the previously mentioned studies of smoking and hearing loss was that smoking status was ascertained by self-report. A direct physical mea-

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measurement of exposure to tobacco smoke would reduce potential problems associated with self-report, such as inaccurate recall, underreporting, and misinterpretation of questions. In addition, assessing exposure to cigarette smoke only in the home probably underestimates total exposure to environmental tobacco smoke among nonsmokers who may be exposed at work or in social settings.

Cotinine, a metabolite of nicotine, is a commonly used biomarker of exposure to tobacco smoke. It has a half-life of approximately 16 to 20 hours and, therefore, reflects tobacco smoke exposure within the past 2 or 3 days. Recent advances in measurement methods have resulted in the ability to detect levels of serum cotinine as low as 0.05 ng/mL (0.28 nmol/L), making it possible to identify exposure to small amounts of environmental tobacco smoke among nonsmokers.

To our knowledge, no studies have examined the relation between serum cotinine level and hearing loss. Consequently, this study assesses the relation between serum cotinine level and incident hearing loss, using a nested case-control study design.

**METHODS**

The Epidemiology of Hearing Loss Study is a population-based study of hearing loss in adults aged 48 to 92 years. From September 15, 1987, to May 4, 1988, residents of Beaver Dam who were aged 43 to 84 years (N = 5924) were identified through a private census and invited to participate in a study of age-related ocular disorders (The Beaver Dam Eye Study, 1988-1990 [N = 4926]). All who participated in the baseline eye examination and were alive as of March 1, 1993, were eligible to participate in the hearing study (Epidemiology of Hearing Loss Study). Of the 4541 eligible adults, 3733 (82.6%) participated, 42.3% of whom were men. The mean age was 65.8 years.

A 5-year follow-up examination was conducted from March 16, 1998, to August 4, 2000. Of 3407 surviving participants, 2800 (82.2%) participated in the 5-year follow-up study. Their mean age at follow-up was 69.3 years, and 41.4% were men.

The Epidemiology of Hearing Loss Study baseline and 5-year follow-up examinations were approved by the Human Subjects Committee of The University of Wisconsin–Madison. Informed consent was obtained from each participant at the beginning of the examinations. A questionnaire about medical history and lifestyle factors, including smoking and exposure to environmental tobacco smoke, was administered as an interview.

Audiologic tests included otoscopy, screening tympanometry, and pure-tone air and bone conduction audiometry. All audiometric and tympanometric equipment complied with American National Standards Institute guidelines. Pure-tone audiometric testing at 250 to 8000 Hz was performed in accordance with recommended American Speech-Language-Hearing Association procedures. Bone conduction testing was conducted at 500 and 4000 Hz at baseline and at 500, 2000, and 4000 Hz at the 5-year follow-up. Audiometers were calibrated every 6 months.

Hearing loss was defined as the pure-tone average of hearing thresholds at 500, 1000, 2000, and 4000 Hz greater than 25-dB hearing level in either ear. Incident hearing loss was defined as hearing loss at the 5-year follow-up that was not present at baseline.

A nested case-control study was conducted among 591 participants to examine the relationship between serum cotinine measurements and incident hearing loss. All 197 case subjects with incident hearing loss who were aged 53 to 75 years were group matched to 394 control subjects using 4 age group–sex strata without regard to smoking status, resulting in a 1:2 case-control ratio.

The serum samples were analyzed by the Air Toxics Laboratory at the Centers for Disease Control and Prevention, Atlanta, Ga, using liquid chromatography–atmospheric pressure ionization tandem mass spectrometry. The limit of detection for this method is 0.05 ng/mL (0.28 nmol/L) of cotinine. Participants with levels below the limit of detection (n = 233) were assigned levels of 0.025 ng/mL (0.14 nmol/L). One participant (a 66-year-old female control) did not have a sufficient volume of serum sample for a conclusive measurement of cotinine, resulting in a missing value.

Fifty randomly selected participants in the case-control study had duplicate samples assayed. The laboratory was masked as to the identity of these participants to assess the reliability of the cotinine measurements. The mean difference between the original (range, 0.025-253 ng/mL [0.14-1437 nmol/L]) and repeat (range, 0.025-263 ng/mL [0.14-1494 nmol/L]) measurements was 0.20 ng/mL (1.14 nmol/L). The Spearman rank correlation coefficient between the original and repeat measurements was 0.97. Restricting the measurements to 15.0 ng/mL or less (≤85 nmol/L) (n = 38 pairs), the mean difference was 0.002 ng/mL (0.01 nmol/L), with a Spearman rank correlation coefficient of 0.92. Further restricting the measurements to those with detectable levels of serum cotinine between 0.05 and 15.0 ng/mL (0.28 and 85 nmol/L) (n = 21 pairs), the mean difference was 0.018 ng/mL (0.10 nmol/L), with a Spearman rank correlation coefficient of 0.97.

Potential confounders and effect modifiers explored included years of education, history of cardiovascular disease (myocardial infarction, stroke, or angina), diabetes mellitus, hypertension (systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or currently taking blood pressure medication), total cholesterol level, high-density lipoprotein cholesterol level, body mass index (calculated as weight in kilograms divided by the square of height in meters), and history of heavy drinking (≥4 alcoholic beverages daily). History of occupational noise exposure, another factor explored, was considered present if the participant (1) had ever had a full-time job that required speaking in a loud voice to be heard by another person for operation. Based on self-report, participants were classified as never smokers (ie, had smoked <100 cigarettes in their lifetime), past smokers, or current smokers. Pack-years was defined as packs of cigarettes smoked per day multiplied by number of years smoked, both based on self-report.

Statistical analyses were conducted with SAS statistical software (SAS Institute Inc, Cary, NC). The Spearman rank correlation coefficient was used to assess the relation between original and repeat measurements of serum cotinine level. The χ² test for general association (for categorical variables) and the t test (for continuous variables) were used to assess differences in participant characteristics by case-control status. The χ² test for general association was used to assess the association between levels of serum cotinine and self-reported smoking status, and between those 2 variables and incident hearing loss. An F test for linear trend was used to assess the association between levels of serum cotinine and mean pack-years. Because a stratified sample was used to select controls rather than one-to-one matching, unconditional logistic regression models were used to evaluate the association between cotinine levels and hearing loss.
Comparisons of participant characteristics by case-control status are shown in Table 1. The mean age of case subjects was 65.2 years; and of control subjects, 64.8 years. The mean total cholesterol level of cases was 212.0 mg/dL (5.48 mmol/L); and of controls, 211.7 mg/dL (5.47 mmol/L). The mean high-density lipoprotein cholesterol level of cases was 53.7 mg/dL (1.38 mmol/L); and of controls, 53.4 mg/dL (1.41 mmol/L). The mean body mass index of cases was 31.4; and of controls, 30.0. Overall, incident hearing loss cases had less education and a higher body mass index than did controls (P<.01). Cases included a lower percentage of smokers and had a higher prevalence of hypertension, although these differences were not statistically significant.

The distribution of serum cotinine level among study participants is shown in Figure 1. Apart from the 233 participants with levels below the limit of detection, the distribution on the log scale is essentially bimodal, with a separation around 10 ng/mL (57 nmol/L). Serum cotinine levels were divided into 5 categories for further analysis. The lowest category represents undetectable levels (coded as 0.025 ng/mL [0.14 nmol/L]). The next 2 categories (0.050-0.500 and 0.501-15.00 ng/mL [0.28-2.84 and 2.85-85 nmol/L]) represent levels typically found among nonsmokers exposed to environmental tobacco smoke, and the highest 2 categories (15.01-190 and 191-588 ng/mL [85-1079 and 1080-3340 nmol/L]) represent levels typically found among smokers.25 Table 2 reveals that serum cotinine levels were strongly associated with mean pack-years of cigarettes smoked and with self-reported smoking status (P<.001). However, there was no association between serum cotinine level or self-reported smoking status and incident hearing loss (Table 3). Multiple logistic regression models were used to further assess the relation between serum cotinine level and incident hearing loss. Exploratory analyses showed that the relation between the logit of the probability of incident hearing loss and serum cotinine measurements was nonlinear, even after a common log transformation was applied to the data. Therefore, modeling cotinine as a continuous measure in a simple linear fashion would have been inappropriate. Although more complex models with polynomial terms were explored, the desire for clarity and interpretability led to the use of the 5 categories of cotinine levels already described, with participants with undetectable levels of cotinine serving as the “unexposed” referent group.

### Table 1. Participant Characteristics by Case-Control Status: Epidemiology of Hearing Loss Study, 1998-2000

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 197)*</th>
<th>Controls (n = 394)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53-64</td>
<td>93 (47.2)</td>
<td>186 (47.2)</td>
</tr>
<tr>
<td>65-75</td>
<td>104 (52.8)</td>
<td>208 (52.8)</td>
</tr>
<tr>
<td>Male sex</td>
<td>100 (54.8)</td>
<td>216 (54.8)</td>
</tr>
<tr>
<td>Smoking status†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>14 (7.3)</td>
<td>41 (10.6)</td>
</tr>
<tr>
<td>Past smoker</td>
<td>96 (49.7)</td>
<td>183 (47.2)</td>
</tr>
<tr>
<td>Never smoker</td>
<td>83 (43.0)</td>
<td>164 (42.3)</td>
</tr>
<tr>
<td>History of heavy drinking†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education, y‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>42 (21.3)</td>
<td>41 (10.4)</td>
</tr>
<tr>
<td>12</td>
<td>102 (51.8)</td>
<td>177 (44.9)</td>
</tr>
<tr>
<td>13-15</td>
<td>28 (14.2)</td>
<td>78 (19.8)</td>
</tr>
<tr>
<td>&gt;16</td>
<td>25 (12.7)</td>
<td>98 (24.9)</td>
</tr>
<tr>
<td>History of hypertension‡</td>
<td>51 (26.1)</td>
<td>55 (14.0)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>22 (11.5)</td>
<td>39 (10.1)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>121 (61.4)</td>
<td>212 (54.1)</td>
</tr>
<tr>
<td>History of occupational noise exposure</td>
<td>117 (59.4)</td>
<td>246 (62.4)</td>
</tr>
<tr>
<td>Serum cotinine level, ng/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetectable</td>
<td>76 (38.6)</td>
<td>157 (39.9)</td>
</tr>
<tr>
<td>0.050-0.500</td>
<td>80 (40.6)</td>
<td>141 (35.9)</td>
</tr>
<tr>
<td>0.501-15.00</td>
<td>18 (9.1)</td>
<td>33 (8.4)</td>
</tr>
<tr>
<td>15.01-190</td>
<td>12 (6.1)</td>
<td>32 (8.1)</td>
</tr>
<tr>
<td>191-588</td>
<td>11 (5.6)</td>
<td>30 (7.6)</td>
</tr>
</tbody>
</table>

**Results**

Figure 1. Distribution of serum cotinine measurements for 590 participants in the incident case-control study, Epidemiology of Hearing Loss Study, Beaver Dam, Wis, 1998-2000. To convert cotinine to nanomoles per liter, multiply by 5.68.

### Table 2. Mean Pack-years Smoked and Self-reported Smoking Status by Serum Cotinine Level: Epidemiology of Hearing Loss Study, 1998-2000

<table>
<thead>
<tr>
<th>Serum Cotinine Level, ng/mL</th>
<th>Mean Pack-years*</th>
<th>Self-reported Smoking Status†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undetectable</td>
<td>12.3</td>
<td>125 (53.9)</td>
</tr>
<tr>
<td>0.050-0.500</td>
<td>14.3</td>
<td>97 (44.9)</td>
</tr>
<tr>
<td>0.501-15.00</td>
<td>21.9</td>
<td>20 (40.0)</td>
</tr>
<tr>
<td>15.01-190</td>
<td>30.4</td>
<td>3 (6.8)</td>
</tr>
<tr>
<td>191-588</td>
<td>38.9</td>
<td>1 (2.6)</td>
</tr>
</tbody>
</table>

SI conversion factor: To convert cotinine to nanomoles per liter, multiply by 5.68.

*Data are given as number (percentage) of each group. Percentages may not total 100 because of rounding and may be “off” because they are based on the total for each characteristic.

†Data were not obtained for 10 participants.

‡Differences were statistically significant (P<.01).
There was no association between levels of serum cotinine and hearing loss in the logistic regression models (Table 4). Education level was the only statistically significant confounding variable to remain in the model. Body mass index was borderline in significance (P = .07), but acted as an effect modifier, so it was also retained in the model. The lack of association between serum cotinine level and hearing loss remained when similar models were stratified by sex, age group, and smoking status (results not shown).

In an effort to explore whether studying higher-frequency changes in hearing status may have led to different results, mean hearing threshold levels at the 5-year follow-up examination were stratified by cotinine level and plotted on an audiogram for all tested frequencies (Figure 2). There was no apparent association between cotinine level and hearing threshold at any of the frequencies. Similarly, there was no apparent association between cotinine level and hearing threshold changes from the previous 5 years at any of the frequencies (data not shown).

This study examined the relation between serum cotinine levels and hearing loss in a cross-sectional, incident, case-control design. No significant associations were found. To our knowledge, no other study has explored the relation between serum cotinine level and incident hearing loss.

These results were not consistent with a previous report, which found cross-sectional associations between hearing loss and current smoking and environmental tobacco smoke exposure in the home.

Several other studies have reported a cross-sectional association between prevalent hearing loss and smoking. Weston studied 408 hearing aid clinic patients with hearing loss and compared them with a sample of 101 elderly people with no complaint of hearing loss. The patients complaining of hearing loss were more than twice as likely to have a history of smoking than were those not complaining of hearing loss. Zelman compared 126 male smokers with 126 male nonsmokers matched by age at a Veterans Administration hospital, and found that hearing thresholds were worse for the smokers than nonsmokers at all frequencies tested (125-12 000 Hz). Bar-
In a longitudinal study\(^8\) of 1554 non–hearing-impaired male office workers, the heaviest smokers were 2.2 times as likely to develop hearing loss at 4000 Hz over 5 years compared with nonsmokers.

Not all studies have found an association between smoking and hearing loss. The Framingham Study\(^7\) assessed low-frequency (250- to 1000-Hz) and high-frequency (4000- to 8000-Hz) hearing loss among 1662 study participants, and found no association. The Baltimore Longitudinal Study of Aging also found no association between baseline smoking habits and the incidence of hearing loss at 500 to 3000 Hz among 531 men, although with only 46 incident cases of hearing loss, there may have been limited power to detect an association.\(^8\)

We explored several possible explanations for the discrepancy between this report and the report by Cruickshanks et al.\(^11\) First, 7.3% of incident cases reported that they stopped smoking between the baseline and 5-year follow-up examination, compared with only 2.6% of controls. It is possible that serum cotinine levels of those who stopped smoking during this period may not accurately reflect the long-term adverse effects of smoking on hearing loss.

Second, more health problems among those smoking at the baseline examination may have contributed to the additional unavailability for follow-up and competing mortality seen with smokers by the 5-year follow-up examination,\(^4\) resulting in fewer smokers participating in the 5-year follow-up examination. It is possible that those smokers who did participate in the 5-year examination were healthier and more resistant to disease (including hearing loss) than those who did not participate.

Third, we explored the possibility that incident cases might have had less cumulative exposure to cigarettes by the time of the baseline examination than did controls. However, an analysis of pack-years of cigarettes smoked revealed no significant differences between incident cases and controls (data not shown).

Among older adults, smoking tends to be a long-term exposure: ie, few adults start smoking for the first time when they are older, and most of those older adults who do smoke have been smoking for many years. It is possible that the previously reported cross-sectional association between smoking and prevalent hearing loss in the baseline examination\(^11\) reflected the long-term effects of smoking on the auditory system, whereas the lack of association between serum cotinine level and incident hearing loss in the present report reflected the difficulty of demonstrating the effects of smoking on the auditory system after a comparatively short 5-year period.

Even though the outcome under study was incident hearing loss, the study was cross-sectional, because serum cotinine measurements were obtained at the 5-year follow-up examination and not at the baseline examination. Given that serum cotinine has a half-life of approximately 16 to 20 hours\(^18\) and, therefore, reflects exposure to tobacco smoke within the past 2 or 3 days, this measure of exposure may not reflect typical exposure levels over a long period.

In summary, this study examined the relation between serum cotinine levels and hearing loss in a cross-sectional, incident, case-control design. No significant associations were found. Future research in this area should focus on longer-term longitudinal relations between smoking and/or serum cotinine levels and the subsequent development of hearing loss.

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