AUGMENTATION OF SMOKING CESSATION EDUCATION
BY URINARY COTININE MEASUREMENT

KUNIHISA MIWA, M.D., YUKO MIYAGI, M.D., HIDETSUGU ASANOI, M.D.
MASatoshi FUJITA, M.D. AND SHIGETAKE SASAYAMA, M.D.

Monitoring the current smoking status by an objective method may augment the effects of smoking cessation instruction. To quantitatively evaluate smoking status and its modification by smoking cessation instruction, urinary cotinine, a major metabolite of nicotine, was measured by radioimmunoassay in 64 patients with a smoking habit before and during smoking cessation instruction. Urinary cotinine levels were used to discriminate between smokers and nonsmokers (with 50 ng/ml used as a threshold). In 49 patients who claimed to have stopped smoking, urinary cotinine concentrations 1 month after instruction indicated that only 30 (61%) of them had actually stopped (before: 243 ±104, after: 1± 3 ng/ml) (mean ± standard deviation). In the remaining 15 patients who failed to stop smoking while reporting a reduction of cigarette consumption (before: 27±12, after: 7±5/day), there was no appreciable reduction in urinary cotinine levels (before: 298 ±140, after: 229±171 ng/ml). When the patients who had failed to stop smoking again received intensive smoking cessation instruction, the success rate increased from 47% (30/64) to 69% (44/64) (p<0.05). Thus, urinary cotinine measurement may provide a useful and quantitative method for monitoring actual smoking habits and thus augment the efficacy of smoking cessation educational programs. (Jpn Circ J 1993; 57: 775—780)

SMOKING is closely related to various human diseases, such as coronary and peripheral vascular disease, chronic obstructive pulmonary disease and cancer; although the precise mechanism has yet to be determined. Although smoking cessation is crucial for reducing the prevalence of these diseases, many patients cannot follow the smoking-cessation program indicated by their physicians. Patients who are unable to successfully quit smoking due to their addiction, may sometimes deceive their physicians by lying about their current smoking status.

Key words:
Smoking
Cotinine
Smoking cessation
Smoking status

(Received November 12, 1992; accepted January 5, 1993)
The Second Department of Internal Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan
Mailing address: Kunihisa Miwa, M.D., 2nd Department of Internal Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

Japanese Circulation Journal Vol.57, August 1993 775

Indeed, data regarding the actual smoking status has depended primarily on information obtained from patients and, therefore, lacked objectivity. Cigarette consumption has been used as a conventional indicator for nicotine intake. However, the amount of nicotine uptake cannot be accurately predicted from the nicotine content of the cigarette or its absorption characteristics because many smokers adjust their smoking behavior to try to regulate or maintain a particular level of nicotine in the body. Thus, it is not surprising to find that the use of low-yield cigarettes has no effect on the risk of some smoking-related diseases, such as coronary artery disease. Recently, Langone et al have shown that cotinine, the major
TABLE I URINARY COTININE CONCENTRATION AND EXCRETION OF SMOKERS AND NONSMOKERS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number</th>
<th>Urinary cotinine concentration (ng/ml)</th>
<th>Urinary cotinine excretion (ng/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>non-smokers</td>
<td>51</td>
<td>2±3 (0—41)</td>
<td>2±4 (0—44)</td>
</tr>
<tr>
<td>smokers</td>
<td>64</td>
<td>245±178 (52—850)</td>
<td>311±260 (57—2117)</td>
</tr>
</tbody>
</table>

Cigarettes smoked per day by smokers

<table>
<thead>
<tr>
<th>Range</th>
<th>Count</th>
<th>Cotinine concentration (ng/ml)</th>
<th>Cotinine excretion (ng/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3—9</td>
<td>6</td>
<td>175±154</td>
<td>242±239</td>
</tr>
<tr>
<td>10—19</td>
<td>25</td>
<td>202±161</td>
<td>298±226</td>
</tr>
<tr>
<td>20—29</td>
<td>15</td>
<td>243±181</td>
<td>281±220</td>
</tr>
<tr>
<td>30—39</td>
<td>10</td>
<td>338±213</td>
<td>390±346</td>
</tr>
<tr>
<td>≥40</td>
<td>8</td>
<td>304±170</td>
<td>350±337</td>
</tr>
</tbody>
</table>

(mean±standard deviation)

metabolite of nicotine, may be a better marker of cigarette smoking than the parent alkaloid, since it has a longer plasma half-life (30 h) and results in a higher concentration in plasma and urine.

In the present study, to quantitatively determine the actual smoking status and the effect of the reduction of cigarette consumption on smoking-related toxin intake, urinary cotinine levels were measured in patients who were advised to stop smoking. Our results suggest that smokers frequently provide false information about their smoking status and that their reported “reduction” in the number of cigarettes smoked does not equate to a reduction in their exposure to toxins.

MATERIALS AND METHODS

The urine samples of 115 consecutive patients (51 nonsmokers (19 males and 32 females, mean age: 56) and 64 smokers (59 males and 5 females, mean age: 53)) who attended our clinic specializing in cardiology, were collected for cotinine measurement. All of the patients were asked about their current smoking habit. All of the smokers were automatically enrolled in our study, although they had attended our clinic for some other reason. They were strongly advised to stop smoking regardless of their actual or suspected disease. Both the risks of smoking and the benefits of quitting were discussed. They returned to our clinic every 2 weeks. At each clinic visit, they were again told to never start smoking again, and were reminded when they continued to stop smoking. Urine samples were collected again 4 weeks after the smoking cessation instruction. However, the subjects were not aware that urine was being collected to test for cotinine that would verify their selfreport.

Urine was stored at −20 °C until assayed. Urine (0.2 ml) was extracted with 2 ml of chloroform, and 0.4 ml of the chloroform extracts were evaporated to dryness in vacuo in duplicate and used for radioimmunoassay. For radioimmunoassay, 125I-l-cotinine (approximately 10,000 cpm), anticotinine antisera from rabbits12 (a final dilution of 1:20,000), and various doses of l-cotinine or dried extracts of the urine samples were mixed and incubated overnight in 0.5 ml of 0.05 M phosphate buffer, pH 7.4, containing 0.1% BSA at 4 °C. After incubation, bound labeled antigen was separated from free labeled antigen by the addition of 0.1 ml of 1% bovine-γ-globulin and 1 ml of 23% polyethylene glycol. After centrifugation at 3000 rpm for 20 min, the supernatant was removed by aspiration and the radioactivity of the precipitate was counted in an automatic gamma spectrometer. The percent bound rate was calculated. Urinary creatinine was also measured, and urinary cotinine excretion was expressed as nanograms per milliliter urine or nanograms per milligram of creatinine to minimize the influence of urine volume.

Statistical analyses

Values are expressed as the mean±stan-
Correlation Between Urinary Cotinine Concentration and Number of Daily Cigarette Consumption

\[ y = 5.05x + 144 \quad (P < 0.05) \]

Fig. 1. Correlation between urinary cotinine concentration and daily cigarette consumption in 64 smokers \((r = 0.31)\).

### TABLE II EFFECTS OF SMOKING CESSATION EDUCATION ON THE URINARY COTININE CONCENTRATION OF 49 SMOKERS WHO CLAIMED TO HAVE STOPPED SMOKING

<table>
<thead>
<tr>
<th>Smoking cessation</th>
<th>Number</th>
<th>Urinary cotinine concentration (ng/ml)</th>
<th>before</th>
<th>after</th>
</tr>
</thead>
<tbody>
<tr>
<td>Success</td>
<td>30</td>
<td>243 ± 104</td>
<td>1 ± 3</td>
<td></td>
</tr>
<tr>
<td>(urinary cotinine ≤ 50 ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fail</td>
<td>19</td>
<td>304 ± 165</td>
<td>298 ± 221</td>
<td></td>
</tr>
<tr>
<td>(urinary cotinine &gt; 50 ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**mean ± standard deviation**

dard deviation. The data regarding daily cigarette consumption, the urinary cotinine concentration and the urinary cotinine creatinine ratio in smokers were subjected to linear-regression analysis. Student’s t-test was used to determine statistical significance. Student’s paired t-test was used to evaluate the effects of reduction of daily cigarette consumption on the urinary cotinine concentration. Proportional data concerning the success rate of smoking cessation were analyzed by the chi-square test. A p value of less than 0.05 was considered statistically significant.

### RESULTS

**Urinary Cotinine Excretion**

Table I shows mean urinary cotinine concentrations and excretions in nonsmokers and smokers. Both the urinary cotinine concentration and excretion were significantly \((p<0.05)\) higher in smokers than in nonsmokers. In the nonsmokers, the urinary cotinine concentration was always below 50 ng/ml, regardless of possible environmental or passive smoking, while in patients who smoked 3 or more cigarettes a day, the concentration was always above 50 ng/ml. The urinary cotinine level roughly correlated to daily cigarette consumption \((r = 0.31, \ p < 0.05, \ \text{Fig.} \ 1 \ \text{and Table} \ 1)\). However, the correlation was not improved when the urinary cotinine excretion or cotinine creatinine ratio was used instead of the urinary cotinine concentration \((r = 0.14, \ p < 0.05)\) (data not shown).

Urine samples were collected 1 month af-
TABLE III  EFFECTS OF REDUCTION OF DAILY CIGARETTE CONSUMPTION ON THE
URINARY COTININE CONCENTRATION OF SMOKERS

<table>
<thead>
<tr>
<th>Smokers</th>
<th>Daily cigarette consumption</th>
<th>Urinary cotinine concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>15</td>
<td>27±12</td>
<td>7±5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>298±140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>229±171</td>
</tr>
</tbody>
</table>

mean ± standard deviation

Urinary Cotinine Concentration Before and After Reducing Cigarette Consumption

Fig.2. Urinary cotinine concentration in patients who failed to stop smoking but reduced their daily cigarette consumption after smoking cessation education.

declaration of smoking cessation was not verified by urinary cotinine measurement, and another 15 patients who reduced their cigarette consumption) were again exposed to intensive smoking cessation instruction. The survey using urinary cotinine measurement performed 1 month later revealed that 14 of them (41%) had successfully stopped smoking, as indicated by urinary cotinine levels of below 50 ng/ml. Of the 14 subjects who successfully quit smoking after the second instruction, 9 had earlier falsely claimed to be nonsmokers (success rate: 47%) and 5 had claimed to have reduced their cigarette consumption (success rate: 33%). Thus, with the aid of urinary cotinine measurement, the success rate of our education program for smoking cessation significantly (p<0.05) increased from 47% (30/64) to 69% (44/64) (Fig. 3).

Fig.3. A flow chart of the results of our smoking cessation program.
DISCUSSION

Cotinine is endogenously produced only through oxidative metabolism of nicotine in the body\(^\text{13}\) Cotinine is more specific for the inhalation of cigarette smoke than blood carboxyhemoglobin, which is formed from hemoglobin on exposure to carbon monoxide. Additionally, cotinine can be more easily identified, since the concentration is higher in urine as a result of a much longer plasma half-life and more protracted urinary excretion rate\(^\text{11,14}\). Therefore, cotinine is considered to be a specific marker for the evaluation for smoking. We chose to examine urinary cotinine because urine is more easily studied than blood in large samples. Our results using urinary cotinine measurement to determine actual smoking status showed that 53% of the subjects failed to stop smoking 1 month after smoking cessation instruction. It is important to note that the declaration of smoking cessation was found to be false in 39% of the subjects.

In the present study, no overlap was observed between the urinary cotinine concentration levels in smokers who habitually smoked more than 3 cigarettes per day and those in nonsmokers. Urinary cotinine has also been suggested to be a sensitive marker of exposure to passive cigarette smoke. The highest urinary cotinine level in nonsmokers with family members who smoked more than 40 cigarettes per day was reported to be nearly identical to that of smokers who smoked less than 3 cigarettes per day\(^\text{12}\). Previous reports have also suggested that simultaneous assays of urinary nicotine and cotinine could be a useful means of verifying a patient's current smoking habits\(^\text{15}\).

The present study failed to show any significant reduction in urinary cotinine concentration in the patients who could not stop smoking but did reduce their cigarette consumption. This may be due to the change in their smoking behavior. The intake of nicotine per cigarette may increase as cigarette consumption is reduced. A previous study indicated that with a reduction from an average of 37 to 5 cigarettes per day, the intake of tobacco toxins per cigarette increased roughly threefold, and daily exposure to tar and carbon monoxide declined only 50 percent\(^\text{9}\). Therefore, the simple recommendation to smoke fewer cigarettes appears to be unsatisfactory unless the smoker eventually quits, because he will compensate by altering his smoking behavior to take in more smoke per cigarette.

Another explanation for the minimal reduction of urinary cotinine concentration in these smokers may involve incorrect self-reported information or a false declaration concerning cigarette consumption. In either of these cases, further education will be needed to achieve complete cessation of smoking in subjects who have reduced their cigarette consumption and in subjects who have failed to stop smoking. Smoking cessation education should be reinforced even for patients who claimed to have stopped smoking, because the success rate of smoking cessation is overestimated, if based on the patient's declaration.

In conclusion, an educational program for smoking cessation can be effectively enhanced by obtaining correct information concerning the current smoking status using urinary cotinine measurement.

Acknowledgments

Anticotinine antisera from rabbits were kindly provided by Prof. Shigeru Matsukura of Miyazaki Medical University, Japan.

This work was supported by a grant-in-aid from the Japan Research Foundation for Clinical Pharmacology.

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Japanese Circulation Journal Vol.57, August 1993