Measurement of Urinary Tobacco Markers in a Smoking-Cessation Program

To the Editor:

To monitor smoking cessation and to motivate subjects trying to stop smoking, we determined nicotine metabolites and thiocyanate in adult men and women attending a group counseling program in a Parisian hospital.

Because noninvasive sampling was preferable for psychological and practical reasons, urine collection was chosen. Nicotine is excreted partially unchanged by the kidney but largely in the form of metabolites that contain an intact pyridine ring and produce colored derivatives when subjected to the simple König reaction (1, 2). Although thiocyanate is not exclusively a product of cigarette smoking (being found in a variety of commonly consumed vegetables), its determination is useful, owing to its slow elimination from body fluids (3), and we measured it with the colorimetric assay from Pettigrew and Fell (4).

For these analyses, urine samples were stored at -20 °C without preservative. Analytical measurements were performed within five days directly in a small urine aliquot in disposable cuvettes. Optical absorbance was determined with a Compact Clinical Analyzer (OLLI C Kone). Concentrations were related to absorbance by the use of known standards of (−)-cotinine (Sigma Chemicals, St. Louis, MO) and potassium thiocyanate (Prolabo, Rhône-Poulenc, France).

Results for the thiocyanate assay varied linearly with concentrations up to 30 μmol/L in the sample. The intersay reproducibility (n = 30) was respectively 5.7% and 5% for 5 and 12 μmol/L thiocyanate standards. The concentration of nicotine metabolites in urine was expressed as "μmol/L cotinine equivalents." Results for this assay varied linearly with concentrations up to 200 μmol/L "cotinine equivalents" in urine. The intersay reproducibility (n = 30) was respectively 8% and 7% for 25 and 85 μmol/L cotinine standards. Interferences were observed in the presence of blood or bile pigments.

A control group (n = 70) consisted of members of the medical staff who declared themselves to be nonsmokers; each one provided a single urine sample. For this group, the mean (SD) concentration of nicotine metabolites was 7.0 (3.8) μmol/L; the range was 1.5–20 μmol/L. The highest values corresponded to subjects exposed to environmental tobacco smoke; their cotinine concentration in urine ranged up to 0.6 μmol/L, as determined with an HPLC technique (5). The urinary thiocyanate mean value (SD) for the control group was 71 (29) μmol/L (range 15–130 μmol/L) (Table 1).

For smoking cessation, the recommended procedure consisted of a series of four visits at weekly intervals, with three follow-up visits at one, three, and six months. Smokers (n = 237) were asked to fill out a questionnaire about their current and past tobacco use habits and medical treatment. They provided a urine sample every time they came to consult: in all, 471 samples were collected. We used a refractometric control to take into account variations of urine concentration over time for a given subject. The smokers' mean (SD) age was 38 (11) years. They started smoking at 18 (SD 5.5) years. Many smokers attended only the initial consultation. For these subjects (72 men, 56 women), the observed upper value of nicotine metabolites was 250 μmol/L; the range of thiocyanate values was 50–460 μmol/L. The mean values for these analytes (Table 1) were significantly different from those found in nonsmokers (P < 0.0001) and reflected the fact that this group included the heavy smokers: 67% of the subjects smoked 20 or more cigarettes a day (mean 23.3, SD 15).

Other smokers (n = 99) attended the consultation two to five times. These subjects (50 men, 49 women) consumed 18.8 (SD 13.6) cigarettes per day; only 53% were smoking more than 20 cigarettes a day. Table 1 documents the decrease of urinary concentration of biochemical markers at each visit. Values of both biochemical markers at entry (1st week) differed significantly from values in nonsmokers (P < 0.0001). Between this group of smokers and the preceding group, concentrations of nicotine metabolites differed significantly (P < 0.01) but those of thiocyanate did not. At the 4th week, nicotine metabolites and thiocyanate values still differed significantly from nonsmokers' values (P < 0.0001 and < 0.02; respectively), but by the fifth week, only nicotine metabolites did (P < 0.02). This latter relation still holds, probably because some patients chew nicotine gum as a help for smoking cessation.

Individual results deserve comments. (For the sake of space, we simply summarize results here, but we will supply detailed case results to readers upon request.) For smokers who stopped smoking immediately after the first visit, nicotine metabolites and thiocyanate showed a simultaneous decrease. A return to the values found in nonsmokers occurred on the second week for nicotine metabolites and on the third week for thiocyanate.

Table 1. Urinary Concentration of Nicotine Metabolites and Thiocyanate (Mean ± SD) in Nonsmokers and in Smokers Attending a Smoking-Cessation Program

<table>
<thead>
<tr>
<th>Nicotine metabolites μmol/L</th>
<th>Measured once</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsmokers</td>
<td>7.0 ± 3.8</td>
<td>81 ± 52</td>
<td>63 ± 38</td>
<td>22 ± 18</td>
<td>19 ± 17</td>
<td>16 ± 14</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>71 ± 29</td>
<td>178 ± 85</td>
<td>166 ± 78</td>
<td>124 ± 52</td>
<td>94 ± 46</td>
<td>85 ± 30</td>
</tr>
<tr>
<td>No. of urine samples</td>
<td>70</td>
<td>128</td>
<td>99</td>
<td>70</td>
<td>53</td>
<td>37</td>
</tr>
</tbody>
</table>

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This is in agreement with the demonstrated half-lives for nicotine-derived metabolites and thiocyanate (3,6). For subjects who stopped smoking with the help of nicotine gum, nicotine metabolites values first decreased, then generally stayed in between 10 and 20 μmol/L as long as the subjects used the gum; thiocyanate values were within the range observed in nonsmokers.

Some smokers (five men and five women), finding it difficult to quit, have come back frequently to group counseling (six to 10 times). For these subjects, cigarette consumption often fluctuates day by day. Variations of the results clearly show the unsuccessful attempts to stop smoking. Nicotine metabolites and thiocyanates rise again to high values, even if the outpatients have smoked fewer cigarettes than before. Sometimes, this kind of intermittent smoking is manifest as apparent discordant results between the two markers.

Our results show that colorimetric assay of nicotine metabolites is able to detect smoking and consumption of nicotine gum. Smokers who become abstainers generally have a lower cigarette consumption and lower concentrations of biochemical markers at entry than those who continue to smoke. This has been similarly observed in determinations of cotinine and thiocyanate in blood (7). These two simple colorimetric assays performed on urine specimens can provide clinicians with objective indicators of patients' smoking behavior. Besides having a favorable psychological impact, these assays can be effectively used to evaluate changes of smoking habits over time.

References

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Unusual Hyperthyroxinemia Caused by Endogenous Thyroxin Antibodies

To the Editor:

Circulating antibodies directed against thyroid hormones have been frequently reported (1–3). Although the thyroid status of patients with these antibodies has varied widely, no specific altered metabolic state directly related to the presence of thyroid hormone autoantibodies has been described. However, these circulating antibodies may interfere with an accurate assessment of the patient's thyroid status in two ways: (a) by increasing the concentrations of circulating thyroxin (T₄) to high values because of excessive binding of T₄ by the antibody, and (b) by altering the measured T₃ through competing with the reagent antibody during the competitive reaction sequence. This latter interference may produce falsely high values (most solid-phase assays) or falsely low values (most single-antibody assays).

We present a case of a clinically asymptomatic 45-year-old woman, whose T₃ concentration was measured as 3999 nmol/L (3100 μg/L). The patient had been diagnosed as hypothyroid with a small goiter in 1961, and had remained euthyroid on thyroid replacement with apparently normal T₄ values for 19 years. In 1986, her T₄ increased to 260 nmol/L (201 μg/L) despite her clinically euthyroid state; however, six years later, she had an increased thyrotropin (TSH) result (in a second-generation TSH assay) suggesting hypothyroidism, despite a T₄ concentration of 735 nmol/L (570 μg/L), and a triiodothyronine (T₃) concentration of 1.37 nmol/L (890 ng/L). At that time she was a healthy woman, with a pulse of 72/min, blood pressure 119/73 mmHg, weight 70 kg, height 160 cm, and no tremor, no exophthalmos, and no lid-lag. Physical examination revealed a symmetrically enlarged thyroid gland, ~3 cm from pole to pole. She was otherwise entirely normal, with no evidence of hypothyroidism. Solely on the basis of the hyperthyroxinemia, familial dysalbuminemic hyperthyroxinemia (FDH) (4) or endogenous antibodies to thyroid hormones were suspected. Appropriate demonstration of excessive T₄ binding to the patient's immunoglobulin by electrophoresis (Paragon agarose gel system; Beckman Instruments, Inc.) confirmed the presence of anti-T₄ autoantibodies, which were probably responsible for the above-normal measurement of T₃ in the serum (Figure 1). The diagnosis of FDH as a genetic abnormality of excessive T₄ binding by albumin was not supported by the normal T₄ value found in her daughter and was excluded on the basis of normal binding of [¹²⁵I]T₄ to albumin (Figure 1). Absence of anti-T₄