ORIGINAL CONTRIBUTION

Limited Value of Urinary Cotinine to Creatinine Ratio as an Indicator for Tobacco Smoke Exposure

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Usefulness of urinary cotinine (COT)/creatinine (Cr) ratio, a widely accepted practical indicator of tobacco smoke uptake, was evaluated in 4 groups of healthy male volunteers (21 in total) with different cigarette doses of 0, 10, 20 and 30 cigarettes per day. 24-hour urine samples were collected on the 7th day of successive controlled smoking to determine urinary COT, thiocyanate (SCN) and Cr concentrations. Although urinary COT concentration exhibited close correlation ($r=0.91$, $p<0.01$) with cigarette dose, this correlation was weakened to $r=0.82$ ($p<0.01$) when it was standardized by urinary Cr concentration. Urinary SCN concentration was, in contrast, unrelated to cigarette dose ($r=0.09$), which however become correlated ($r=0.71$, $p<0.05$) after Cr standardization, in part due to the variation of urinary Cr concentration which was found inversely linked to cigarette dose ($r=-0.47$, $p<0.05$). Multiple regression analyses revealed that extent of urine volume-dependent decrease in urinary COT concentration was of several orders smaller in magnitude than that of urinary SCN or Cr concentrations as estimated on the basis of standardized partial regression coefficients. These results suggested that urinary COT/Cr ratio, compared to urinary COT concentration, tends to exaggerate the actual smoke uptake in the individuals with increased urine flow as well as in the smokers with smoking-associated decrease in urinary Cr concentration. J Epidemiol, 1992; 2: 91-95.

tobacco smoke, cotinine, thiocyanate, creatinine ratio

Accurate assessment of tobacco smoke uptake is important since tobacco smoke has been incriminated to various hazardous health conditions not only of active smokers but of individuals involuntarily exposed to environmental tobacco smoke¹⁻³. Among a variety of biological indicators of smoke uptake, cotinine (COT)⁴, a major metabolite of nicotine, has been regarded most reliable because of its specificity to tobacco smoke, longer circulating half life⁵ compared to its parental compound, nicotine, and because it is readily measurable by a sensitive radioimmunoassay⁶ or gas chromatography⁷, inspite of a great interlaboratory variation in the measurement⁸. COT is particularly useful in assessing the extent of environmental tobacco smoke uptake since it is concentrated in urine and well correlates with self-reported degree of tobacco smoke exposure⁹⁻¹¹. As a biological sample for COT determination, single-spot urine instead of 24-hour urine has been used for practical reasons and urinary COT excretion is sometimes expressed in the form of creatinine (Cr) ratio, expecting to adjust for the variable COT dilution by urine flow¹². Irrespective of such a practical importance of COT/Cr ratio as an indicator of smoke uptake, validation for its use has as yet been, to our knowledge, fully established.

We here present the results of a controlled smoking experiment in men and suggest that COT/Cr ratio is useful only on a limited basis.

MATERIALS AND METHODS

Twenty one healthy young male volunteers aged

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20-30 years of non-smoker or current smoker were recruited from a class of medical students. They were allocated into 4 groups (5 or 6 for each) according to the self-reported mean daily cigarette consumption, 0, 10, 20 and 30 cigarettes per day. Ages were 28.6 ± 1.9, 21.5 ± 2.3, 23.8 ± 1.9 and 23.4 ± 2.1 (years, Mean ± SD) and their body sizes in Quetlet's index were 21.7 ± 1.0, 23.7 ± 2.8, 21.7 ± 1.3 and 21.0 ± 1.1 (kg/m²) without statistical significance between the groups except for the age of the non-smoking group which was significantly higher than smoking groups. Blood pressure and vital capacity of the subjects measured before the study were identical without significant difference between the groups. 10, 20 and 30 filter-tipped cigarettes of the same brand (Seven Star, Japan Tobacco Co. Ltd., Tokyo, Japan) containing 0.9 mg of nicotine were given to the members of the respective smoking groups everyday for 7 days. No restriction other than to consume all cigarettes given and to smoke two-thirds length of each cigarette was placed in the rest of daily life including the depth of each puff, number of puffs per cigarette, diet or fluid intake. On the 7th day of the study 24-hour urine specimens were collected including from the second voided urine of the 7th day morning to the first voided urine before breakfast on the next morning using a portable urine collecting device, Aliquot Cup. All subjects were confirmed for the completeness of urine collection by direct interview. Blood samples were collected in the next morning of the 7th day from the subjects who fasted and refrained from smoking overnight to separate plasma. Plasma and urine samples were stored at -70°C until measurement. COT levels in plasma and urine were determined by a radioimmunoassay according to the procedures described elsewhere and SCN according to the method of Butts et al. using an automated analysing apparatus (Autoanalyzer II, Technicon Instruments Corp., Tarrytown, NY).

Data are expressed as mean ± SD. Difference in group means was tested by one way analysis of variance (ANOVA) and correlation between parameters were analysed by simple regression analysis as well as by multiple regression analysis.

RESULTS

In Table 1 listed are mean ± SD of COT-, SCN- and Cr-related variables in plasma or urine of each group.

| Table 1. Plasma and Urinary Concentrations of Cotinine, Thiocyanate and Creatinine. |
|---|---|---|---|---|---|
| Variables | 0 (n=5) | 10 (n=6) | 20 (n=5) | 30 (n=5) | Result of ANOVA |
| **Cotinine** | | | | | |
| Plasma concentration (ng/ml) | 17.0 ± 2.4 | 62.5 ± 44.0 | 156 ± 48 | 200 ± 102 | p < 0.01 |
| Urinary concentration (ng/ml) | 40.0 ± 4.5 | 360 ± 89 | 704 ± 137 | 800 ± 182 | p < 0.01 |
| Creatinine ratio (ng/mg) | 26.1 ± 7.1 | 206 ± 71 | 511 ± 94 | 790 ± 378 | p < 0.01 |
| Total urinary output (µg/day) | 44.3 ± 10.9 | 310 ± 101 | 745 ± 174 | 1116 ± 372 | p < 0.01 |
| **Thiocyanate** | | | | | |
| Plasma concentration (ng/ml) | 18.8 ± 5.0 | 46.3 ± 21.2 | 82.8 ± 12.7 | 87.6 ± 13.9 | p < 0.01 |
| Urinary concentration (µmol/l) | 137 ± 26 | 135 ± 18 | 153 ± 34 | 138 ± 26 | p = 0.74 |
| Creatinine ratio (10⁶ µmol/mg) | 85.0 ± 8.8 | 79.3 ± 15.1 | 110 ± 18 | 127 ± 17 | p < 0.01 |
| Urinary total output (µmol/day) | 145 ± 12 | 130 ± 34 | 159 ± 28 | 186 ± 15 | p < 0.05 |
| **Creatinine** | | | | | |
| Plasma concentration (mg/dl) | 0.92 ± 0.13 | 0.98 ± 0.11 | 0.96 ± 0.10 | 1.08 ± 0.08 | p = 0.20 |
| Urinary concentration (mg/ml) | 1.65 ± 0.44 | 1.74 ± 0.29 | 1.43 ± 0.40 | 1.13 ± 0.36 | p = 0.13 |
| Total urinary output (g/day) | 1.71 ± 0.08 | 1.56 ± 0.16 | 1.47 ± 0.25 | 1.49 ± 0.18 | p = 0.22 |
| Urine volume (l/day) | 1.11 ± 0.26 | 0.92 ± 0.18 | 1.13 ± 0.43 | 1.40 ± 0.30 | p = 0.17 |

Mean ± SD
Significant dose-dependent increase was observed in all COT- and SCN-related variables except for urinary SCN concentration which remained almost constant irrespective of cigarette dose. Coefficients of variation (CV) was found smallest in urinary Cot concentration (11.2 - 24.8%) among COT-related variables, while it was smallest in urinary total output (8.2 - 26.2%) or Cr ratio (10.1 - 19.0%) among SCN-related variables. Dose-dependent decrease was detected in urinary Cr concentration and urinary total Cr output, while urine volume tended to increase with increasing cigarette consumption, all of which however did not reach statistical significance by ANOVA. The present data have been highly suggestive of environmental tobacco smoke exposure to our non-smoking subjects since smoker/non-smoker ratios of urinary COT concentration and COT/Cr were at the largest about 20 and 30, respectively, which were much smaller than the reported values in the literature.

Results of simple regression analyses are summarised in Table 2. In COT, urinary concentration and total urinary output were closely correlated with cigarette dose, while the correlation was slightly diminished in COT/Cr ratio. In contrast, although urinary concentration and total urinary output of SCN were little or poorly correlated with cigarette consumption, correlation was much improved in SCN/Cr ratio. Such an increase in correlation by expressing urinary SCN excretion in Cr ratio is in part or completely explained by a confounding effect of urinary Cr concentration which was found in a significant inverse relationship with cigarette dose. Decreased correlation by Cr ratio of COT, on the other hand, appeared further confounded by the effect of urine volume since urine volume-dependency of urinary concentration was equivalent between SCN and Cr, which was however much smaller in COT. Since cigarette dose, urine volume and urinary concentration of COT are mutually correlated, multiple regression analysis was performed to clarify and quantify their interrelationships.

Results of multiple regression analyses are listed in Table 3, together with the results of SCN and Cr for comparison. These analyses revealed that urinary COT concentration decreased in a dose-dependent manner with increasing urine volume, extent of which

| Table 2. Results of Simple Regression Analyses. |
|------------------------------------------------|
|                     | Simple correlation coefficients |                     |
|                     | Number of cigarettes smoked per day | 24-hour urine volume |
| **Cotinine**        |                              |                     |
| Plasma concentration| 0.76**                        | 0.27               |
| Urinary concentration| 0.91**                       | 0.15               |
| Creatinine ratio    | 0.82**                        | 0.48*              |
| Total urinary output| 0.88**                        | 0.57*              |
| **Thiocyanate**     |                              |                     |
| Plasma concentration| 0.85**                        | 0.25               |
| Urinary concentration| 0.09                         | -0.72**            |
| Creatinine ratio    | 0.71**                        | 0.56**             |
| Total urinary output| 0.53*                        | 0.74**             |
| **Creatinine**      |                              |                     |
| Plasma concentration| 0.41                          | 0.21               |
| Urinary concentration| -0.47*                       | -0.89**            |
| Total urinary output| -0.42                        | 0.20               |
| **Urine volume**    | 0.36                          | 1.00               |

Statistical significance * : p<0.05, ** : p<0.01

| Table 3. Results of Multiple Regression Analyses. |
|------------------------------------------------|
| Independent variables | Partial regr. coeff. (Standard partial regr. coeff.) |
|-----------------------|---------------------------------------------------|
|                       | Urinary COT concentration | Urinary SCN concentration | Urinary Cr concentration |
| **Cigarette dose**    | 28.2 (0.97)**              | 0.98 (0.39)*              | -0.007 (-0.17)*          |
|                       | 0.92                      | 0.53                      | -0.36                    |
| **Urine volume**      | -174 (0.19)*              | -67.2 (-0.86)**           | -1.05 (-0.83)**          |
|                       | -0.42                     | -0.80                     | -0.88                    |
| **Regression constant**| 240                        | 202                       | 2.79                     |
| **Multiple regr. coeff.** | 0.92                    | 0.80                      | 0.90                     |
| **F value**           | 46.3**                    | 16.6**                    | 40.4**                   |

1) Regr. coeff. = regression coefficient, COT = cotinine, SCN = thiocyanate
2) Pooled means±SD of variables of all subjects are as follows: cigarette dose (number smoked per day)=14.8±11.0, urine volume (l/day)=1.13±0.35, urinary cotinine concentration (ng/ml)=482±320, urinary thiocyanate concentration (µmol/l)=140±27, urinary creatinine concentration (mg/ml)=1.50±0.44.
3) Partial correlation coefficients are presented in italic.
4) Statistical significance * : p<0.05, **p<0.01, a : p=0.08, b : p=0.13
was however only slight, approximately 1:4.5 of urinary SCN and Cr concentrations on the basis of standard partial regression coefficients. It was also noted that urinary SCN concentration was in a positive correlation with cigarette dose, which was unable to detect in the simple regression analysis because of the confounding effect of urine volume.

These results altogether suggested that the use of urinary COT/Cr ratio is limited compared to urinary COT concentration since it could be confounded by the cigarette dose-dependent decrease in urinary Cr concentration as well as by the urine volume-dependency of urinary COT concentration which was unproportionally small compared to that of urinary Cr concentration.

DISCUSSION

The rationale for using Cr ratio as an index for estimating urinary excretion of micronutrients or their metabolic end products is based upon the assumptions (1) that daily Cr excretion is constant and (2) that urinary component is volume-dependently diluted by urine. Regarding the first assumption, it has been well recognized that there is a definite day-to-day variation in Cr excretion as well as the diurnal variation where the Cr excretion is lowest during early morning and highest in the afternoon16). Body size- or age-dependency of urinary Cr excretion has been well established as one of the causes of inter-individual variation of Cr excretion16,17). Our present results demonstrated that urinary Cr concentration correlated inversely with cigarette consumption in accordance with the observation of Adlkofer et al.18), suggesting a further potential source of variation in using Cr ratio in clinical or populational surveys. Difference in mean urinary Cr concentrations between non- or light smokers and heavy smokers was as great as more than 30% in the present study, similar to the values reported by Adlkofer et al.18) which was about 40% between the light smokers (smoking less than 10 cigarettes/day) and heavy smokers (smoking more than 30 cigarettes/day). Our finding is unlikely confounded by the influence of body size or blood pressure17) between the study groups since neither body mass index nor blood pressure was related to cigarette consumption. From our results decreased urinary Cr concentration may be explained by a combined effect of decreased total urinary Cr output and the greater urine volume particularly in heavy smokers consuming 30 cigarettes per day. Although slightly reduced plasma Cr levels for smokers as compared with non-smokers were reported by some investigators19,20), which could be a potential cause of lower urinary Cr concentration in smokers, such was not confirmed in the present study consistent with other authors21). Greater urine volume observed in the group smoking 30 cigarettes per day is difficult to simply explain by an effect of heavy smoke exposure since intake of fluid or diet was not controlled in the present study, which however might be an effect of smoking since accelerated urine flow by smoking was reported by other authors22).

Regarding the second assumption, multiple regression analysis in the present study revealed that urinary COT concentration was much less diluted by urine volume compared to urinary SCN and Cr concentrations. This finding suggests that expressing urinary COT excretion by means of Cr ratio could introduce rather than adjust the variation due to the inter- or intra-individual difference in urine volume which could be influenced by a variety of factors in daily life. In a occasion where urinary Cr concentration is decreased due to the increased urine flow, COT/Cr ratio may lead to an exaggerated estimation of actual tobacco smoke exposure.

From the present results it is concluded that urinary COT concentration may be more appropriate than COT to Cr ratio as a urinary index for tobacco smoke exposure contrary to what had been suggested12). This would be so even for the assessment of environmental tobacco smoke exposures since variability of COT/Cr in terms of CV was twice greater than that of urinary COT concentrations in non-smoker group (27.2% vs 11.2%) and would be particularly so in the studies employing random spot urine samples where accurate informations on factors which influence the urine flow of individuals are hardly collectable. Finally, the present findings might be extended to more generalized conclusion that possible influence of tobacco smoking on urinary Cr concentration could be one of the potential confounding factors in the investigations which use Cr ratio of urinary components and involve both non-smokers and smokers and that magnitude of urinary dilution require to be evaluated for any urinary constituent before it is standardized by urinary Cr concentration in clinical or epidemiological surveys.

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