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Creatinine at the Evaluation of Urinary 1-Methyladenosine and Pseudouridine Excretion

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Honda, I., Itoh, K., Mizugaki, M. and Sasaki, T. *Creatinine at the Evaluation of Urinary 1-Methyladenosine and Pseudouridine Excretion.* Tohoku J. Exp. Med., 1999, **188** (2), 133–138 —— The elevation of urinary modified nucleosides levels in urine is found in patients with cancers. In the precent study, we have tested 616 urine samples randomly collected from non-malignant cases. Thirty-two percent (194/616) and 11% (68/616) had elevated levels of 1-methyladenosine and pseudouridine, respectively (They are designated as false-positive cases). To elucidate the cause on non-specific elevation of the nucleosides, the correlation between creatinine excretion level and urinary nucleosides levels were determined. The result revealed that false-positive cases were frequently detected in patients with lower creatinine excretion levels. The mean creatinine levels of false-positive cases were significantly lower than those of negative cases. From these results, the false-positive of urinary 1-methyladenosine and pseudouridine might be due to the low creatinine excretion mainly caused by the renal dysfunction. Creatinine excretion in each individual should be taken into consideration in case of determining urinary modified nucleosides. —— modified nucleosides; 1-methyladenosine; pseudouridine; creatinine excretion

All types of RNAs contain modified nucleosides which are post-transcriptionally introduced into these molecules. Most modified nucleosides are not reutilized but excreted in urine as intact forms.

So far many studies have reported that elevated levels of the modified nucleosides in urine were detected in various types of cancer patients (Waalkes et al. 1975; Davis et al. 1977; Gehrke et al. 1979; Fishbein et al. 1983; Rasmuson et al. 1983). We have also reported that increased 1-methyladenosine and pseudouridine levels were detected in patients with leukemia and lymphoma (Itoh et al.

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ELISA processor II (Hoechst, Frankfurt, Germany). Briefly, wells of polystyrene microplates (MS-3496F; Sumitomo Bakelite, Tokyo) were fixed overnight at 4°C with a 1-methyladenosine-BSA conjugate (10 μg/ml) or with a pseudouridine-BSA conjugate (10 μg/ml) dissolved in PBS. The wells were washed once with PBS and filled with 100 μl of 1% BSA in PBS. After 1 hour incubation at 37°C, the solution was discarded, and 50 μl of serially diluted authentic 1-methyladenosine, authentic pseudouridine, or adequately diluted urine sample was added to each well. An equal volume of 1 μg/ml anti-1-methyladenosine monoclonal antibody (AMA-2) or 5 μg/ml anti-pseudouridine monoclonal antibody (APU-6) was then added, and the reaction mixture with incubated for 1 hour at 4°C. The wells were washed 5 times with PBS and filled with 100 μl of 1 : 3000 diluted alkaline phosphatase (ALP)-labeled goat anti-mouse IgG (TAGO, Burlingame, CA, USA) followed by an incubation for 45 minutes at 4°C. After the similar washing procedure was done, 100 μl of p-nitrophenyl phosphate (104 phosphatase substrate, Sigma, St. Louis, MO, USA) in 1 M diethanolamine buffer (pH 9.8) was added, and the reaction mixture was incubated for 30 minutes at 37°C. The absorbance of developed yellow color in each well was measured at 405 nm. The urinary concentrations of 1-methyladenosine and pseudouridine were interpolated from the standard curve, and expressed as a function of creatinine (nmol nucleoside/μmol creatinine). The creatinine concentration in each sample was determined by Creatinine Test Wako (Wako Pure Chemical, Osaka).

Statistical analysis

The χ^2-test was used for determination of the significance of differences.

Results

To elucidate the cause of non-specific increase of 1-methyladenosine and pseudouridine in non-malignant cases, we determined the correlation between these nucleosides levels (expressed as a function of creatinine)(nmol nucleoside/μmol creatinine) and the creatinine concentration (μmol/ml). As shown in Figs. 1 and 2, higher values of 1-methyladenosine and pseudouridine were detected more frequently in patients that showed lower creatinine excretion levels. The mean creatinine excretion levels of the tested patients in relation to the cut-off values of 1-methyladenosine and pseudouridine are shown in Table 2. Cut-off values for 1-methyladenosine (3.23 nmol/μmol creatinine) and pseudouridine (51.04 nmol/μmol creatinine) were established in our previous experiments (Itoh et al. 1988, 1989). Urinary creatinine excretion of patients who showed 1-methyladenosine levels above the cut-off value (6.54 ± 4.07 μmol/ml) was significantly lower than those within normal levels of 1-methyladenosine (9.33 ± 5.92 μmol/ml) ($p < 0.05$) (Table 2). Urinary creatinine excretion of the patients who showed pseudouridine levels above the cut-off value (6.53 ± 3.80 μmol/ml) was also

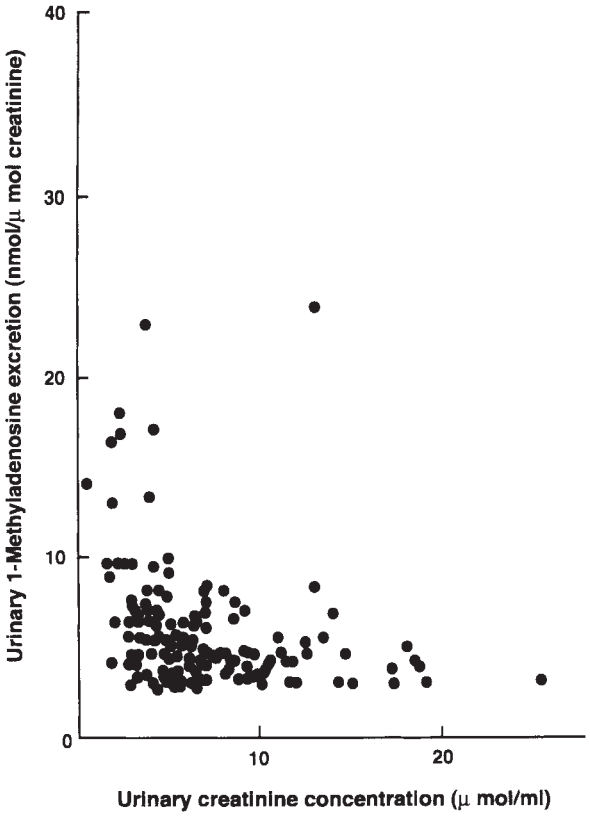

Fig. 1. Correlation between creatinine excretion and urinary levels of 1-methyladenosine in non-malignant cases.

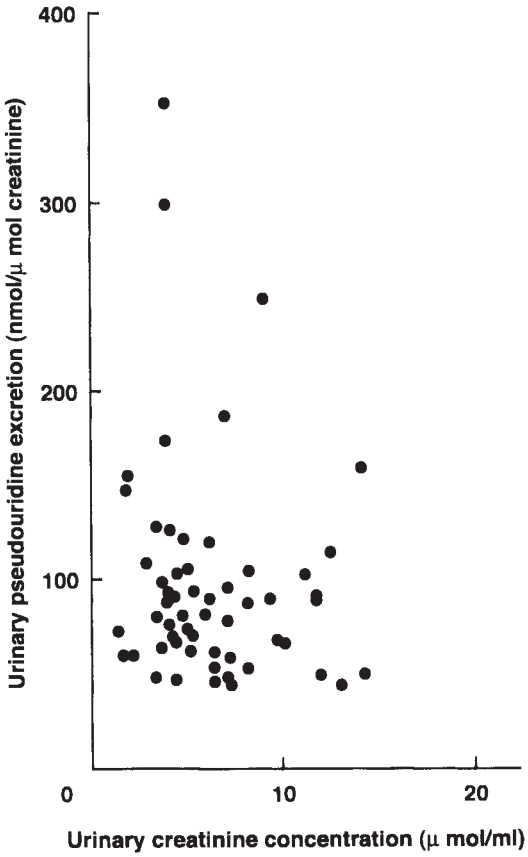

Fig. 2. Correlation between creatinine excretion and urinary levels of pseudouridine in non-malignant cases.

TABLE 2. *Relationship between urinary nucleosides and creatinine levels*

Nucleosides	(nmol/μmol creatinine)	Number of specimen	Creatinine (μmol/ml)
1-Methyladenosine	$\geqq 3.23$[a]	194	6.54 ± 4.07*
	< 3.23	422	9.33 ± 5.92
Pseudouridine	$\geqq 51.04$[a]	68	6.53 ± 3.80*
	< 51.04	547	8.71 ± 5.73

[a]Cut-off value was set at the normal mean $\pm$ 2 S.D.
*$p < 0.05$ as compared to the group below cut-off value.

significantly lower than those within normal levels of pseudouridine (8.71 ± 5.73 μmol/ml)($p < 0.05$). Ninety six (48%) among 200 false-positive cases had renal and urogenital diseases, and patients with severe infectious diseases (8.5%; 17/200) or those with cardiovascular diseases (7.5%; 15/200) had moderately high false-positive rates (Table 2).

DISCUSSION

Creatinine is one of the normal components in urine, and its output is directly related to muscle mass. Gehrke et al. (1979) reported that nucleoside/creatinine ratio in spot urine samples is similar to that in the total urine obtained during 24 hours. They, therefore, concluded that the spot urine samples will be satisfactory sources for the determination of modified nucleosides. As for 1-methyladenosine and pseudouridine, we have determined the age and sex differences, variation within a day, and variation between days in healthy individuals, and have confirmed their results (Itoh et al. 1993).

In our preliminary analysis, false-positive cases frequently appeared in low creatinine output cases (Figs. 1 and 2), and 48% of false-positive cases had renal diseases (Table 1). Normal creatinine output would be the essential factor for the accurate determination of modified nucleosides in spot urine samples. In measuring the modified nucleosides levels in cancer patients, the following should be taken into consideration; 1) The patients with the diseases such as renal dysfunction, infectious diseases, or cardiovascular diseases, that may affect the creatinine excretion should be excluded. 2) Infants should also be excluded because only small amount of urine is available (Heldman et al. 1983). 3) The creatinine concentration should be reconsidered when extremely high modified nucleosides excretion levels are detected.

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References

1) Borek, E., Baliga, B.S., Gehrke, C.W., Kuo, K.C., Belman, S., Troll, W. & Waalkes, T.P. (1977) High turnover rate of transfer RNA in tumor tissue. *Cancer Res.*, **37**, 3362-3366.
2) Davis, G.E., Suits, R.D., Kuo, K.C., Gehrke, C.W., Waalkes, T.P. & Borek, E. (1977) High performance liquid chromatographic separation and quantitation of nucleosides in urine and some other biological fluids. *Clin. Chem.*, **23**, 1427-1435.
3) Fishbein, A., Sharma, O.K., Selikoff, I.J. & Borek, E. (1983) Urinary excretion of modified nucleosides in patients with malignant mesothelioma. *Cancer Res.*, **43**, 2971-2974.
4) Gehrke, C.W., Kuo, K.C., Waalkes, T.P. & Borek, E. (1979) Patterns of urinary excretion of modified nucleosides. *Cancer Res.*, **39**, 1150-1153.
5) Heldman, D.A., Grever, M.R., Miser, J.S. & Trewyn, R.W. (1983) Relationship of urinary excretion of modified nucleosides to disease status in childhood acute lymphoblastic leukemia. *J. Natl. Cancer Inst.*, **71**, 269-273.
6) Itoh, K., Mizugaki, M. & Ishida, N. (1988) Preparation of a monoclonal antibody specific for 1-methyladenosine and its application for the detection of elevated levels of 1-methyladenosine in urines from cancer patients. *Jpn. J. Cancer Res. (Gann)*, **79**, 1130-1138.
7) Itoh, K., Mizugaki, M. & Ishida, N. (1989) Detection of elevated amounts of urinary pseudouridine in cancer patients by use of a monoclonal antibody. *Clin. Chim. Acta*, **181**, 305-316.
8) Itoh, K., Konno, T., Sasaki, T., Ishiwata, S., Ishida, N. & Mizugaki, M. (1992) Relationship of urinary pseudouridine and 1-methyladenosine to activity of leukemia and lymphoma. *Clin. Chim. Acta*, **206**, 181-189.
9) Itoh, K., Aida, S., Ishiwata, S., Sasaki, S., Ishida, N. & Mizugaki, M. (1993) Urinary excretion patterns of modified nucleosides, pseudouridine and 1-methyladenosine, in healthy individuals. *Clin. Chim. Acta*, **217**, 221-223.
10) Rasmuson, T., Bjork, G.R., Damber, L., Holm, S.E., Jacobson, L., Jappson, A., Littbrand, B., Stigbrand, T. & Westman, G. (1983) Evaluation of carcinoembrionic antigen, tissue polypeptide antigen, placental alkaline phosphatase, and modified nucleosides as biological markers in malignant lymphomas. *Recent Res. Cancer Res.*, **84**, 331-343.
11) Schoch, G. & Heller-Schoch, G. (1977) Molekularbiologie und klinische Bedeutung des Staffwechsels normales und modifizierter Nucleobasen. *Helv. Pediatr. Acta*, Suppl., **38**, 7-171.
12) Waalkes, T.P., Gehrke, C.W., Zumwalt, R.W., Chang, S.Y., Lakings, D.B., Tormey, D.C., Ahmann, D.L. & Moertel, C.G. (1975) The urinary excretion of nucleosides of ribonucleic acid by patients with advanced cancer. *Cancer*, **36**, 390-398.