

# Possible Prediction of Adverse Reactions to Pyrimidine Chemotherapy from Urinary Pyrimidine Levels and a Case of Asymptomatic Adult Dihydropyrimidinuria<sup>1</sup>

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## ABSTRACT

**Deficiency of dihydropyrimidine dehydrogenase or dihydropyrimidinase, enzymes that catalyze the breakdown of pyrimidine chemotherapy agents such as 5-fluorouracil, may cause serious adverse reactions to these agents. We attempted to establish the reference range for urinary pyrimidines in adults to detect individuals with abnormal pyrimidine metabolism.**

**We analyzed urinary pyrimidine levels in 1133 adults to establish a reference range for persons ages 20 years or older. Urinary dihydrouracil and uracil levels were determined by high-performance liquid chromatography with column switching.**

**The reference range obtained was found to be 0-59.3  $\mu\text{mol/g}$  creatinine for dihydrouracil and 0-129.8  $\mu\text{mol/g}$  creatinine for uracil. In addition, an asymptomatic man with suspected dihydropyrimidinase deficiency was detected on the basis of dihydropyrimidinuria. Although only three cases of this disease have been found worldwide, including one infant reported previously by our group, it may not be so rare as has been thought. In this man, a 10 mg/kg oral uracil loading test yielded a peak blood dihydrouracil level of 192.1  $\mu\text{mol/liter}$  and a peak uracil level of 67.8  $\mu\text{mol/liter}$ . Eight h after loading, the uracil level was still 11.1  $\mu\text{mol/liter}$ , about 17 times that in healthy subjects.**

**Additional research on dihydropyrimidinase deficiency may help to prevent adverse reactions to pyrimidine chemotherapy agents in susceptible individuals.**

## INTRODUCTION

Pyrimidine chemotherapy agents such as 5-FU<sup>3</sup> are used widely but can occasionally cause serious adverse reactions. In pyrimidine metabolism, uracil and thymine are first converted to dihydropyrimidines (dihydrouracil and dihydrothymine) by DPD and then are metabolized to  $\beta$ -ureidopropionic acid and  $\beta$ -ureidoisobutyric acid, respectively, by dihydropyrimidinase. These compounds are converted subsequently into  $\beta$ -alanine and  $\beta$ -aminoisobutyric acid, respectively (1). Because about 80% of an administered dose of 5-FU is degraded by this pathway, there is a possibility of adverse reactions occurring as a result of DPD or dihydropyrimidinase deficiency. The value of measuring peripheral blood monocyte DPD activity before administering 5-FU has been suggested in Western countries (2-6), but DPD deficiency has not been reported in Japan.

Using a method that permits analysis of dihydrouracil and uracil in small volumes of urine, we previously screened Japanese infants for abnormalities of pyrimidine metabolism and detected a case of infantile dihydropyrimidinuria (7). In the present study, we used the same method to measure urinary pyrimidines in 1133 Japanese adults and attempted to establish reference ranges for urinary dihydrouracil and uracil.

## PATIENTS AND METHODS

Urinary pyrimidines were measured in a total of 1133 subjects ages 20 years and older, who gave informed consent to participation in this study. They included 966 patients examined at Nagoya City Higashi General Hospital and 167 persons who underwent a screening examination or were healthy volunteers. The urinary levels of dihydrouracil and uracil were determined as well as their ratio. We excluded pregnant women and patients who were being treated with 5-FU or its derivatives.

To investigate the possible effect of food intake, urinary dihydrouracil and uracil levels were measured in the first morning urine of 76 subjects, in the second urine of 76 subjects, and in urine collected randomly from 165 subjects.

Fifty-seven of the patients had abnormal hepatic or renal function and were studied to evaluate the relationship between urinary pyrimidine levels and hepatorenal function.

Pyrimidines were measured after separating urinary sediments by centrifugation and passing the serum through filters to separate proteins only. These were analyzed directly by high-performance liquid chromatography with column switching (8-10), and urinary Cre was measured with an autoanalyzer (TBA80FR; Toshiba). The identity of dihydrouracil and uracil was confirmed by gas chromatography with mass spectrometry (7).

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<sup>3</sup> The abbreviations used are: 5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; Cre, creatinine.

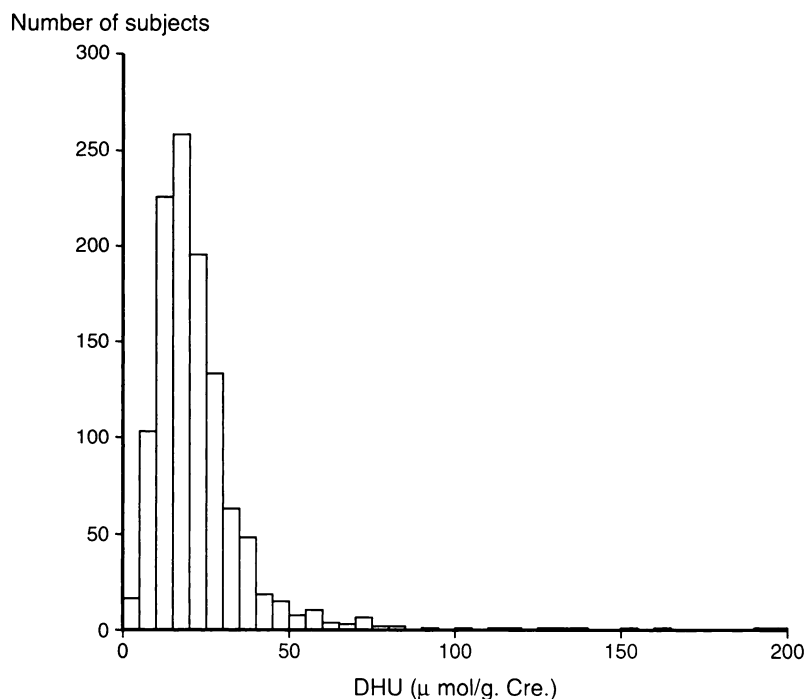
Table 1 Urinary pyrimidine concentrations in morning urine and random urine<sup>a</sup>

	DHU <sup>b</sup> (μmol/g Cre)	Uracil (μmol/g Cre)	DHU/uracil
First early morning urine (n = 76)	18.7 ± 14.4	54.1 ± 42.5	0.39 ± 0.25
Second early morning urine (n = 76)	18.8 ± 14.2	55.6 ± 31.1	0.39 ± 0.38
Random urine (n = 165)	17.5 ± 5.5	66.1 ± 30.0	0.31 ± 0.16

<sup>a</sup> All values are shown as mean ± SD.

<sup>b</sup> DHU, dihydrouracil.

Fig. 1 Distribution of urinary dihydrouracil levels in the 1133 subjects. The mean ± SD was 23.81 ± 35.52 μmol/g Cre. The mean ± 1 SD ranged from 0 to 59.3 μmol/g Cre.



For statistical processing, we used RANS (Version 4.0), using the Student *t* test.

## RESULTS

The mean age of the subjects was 58.7 ± 18.3 years (range, 20–98 years), and there were 624 men and 509 women. Six hundred twenty-four subjects were ages 60 years or older, and 509 were less than 60 years of age. The diagnosis was hypertension in 216 patients, cerebral infarction in 135 patients, malignancy in 51 patients, liver dysfunction in 50 patients, and nephropathy in 7 patients.

**Urinary Concentrations.** Urinary dihydrouracil and uracil levels were measured in the first morning urine of 76 subjects, in the second urine of 76 subjects, and in urine collected randomly from 165 subjects (Table 1). Because there was no significant difference among the results, the normal ranges were determined using all of the random measurements.

The mean ± SD urinary dihydrouracil level was 23.81 ± 35.52 μmol/g Cre (Fig. 1; the mean ± 1 SD ranged from 0 to 59.3 μmol/g Cre). A dihydrouracil level of 59 μmol/g Cre or higher was found in 20 subjects with the following diagnoses: 4 with hypertension; 2 with liver cirrhosis; 2 with vaginitis; and 1

each with heart failure, malignant lymphoma, low back pain, chronic rheumatoid arthritis, gastric ulcer, cerebral infarction, esophageal ulcer, lung cancer, dehydration, prostatic hypertrophy, urinary calculi, and renal failure.

The mean ± SD urinary uracil level was 63.81 ± 66.00 μmol/g Cre (Fig. 2; the mean ± 1 SD ranged from 0 μmol/g Cre to 129.8 μmol/g Cre). A uracil level of 130 μmol/g Cre or higher was found in 29 subjects.

The diagnoses of these subjects were as follows: cystitis, cerebral infarction, and hypertension in three patients each; urethritis, vaginitis, renal failure, uterine myoma, dehydration, and prostatic hypertrophy in two patients each; and heat stroke, hyperthermia, prostatic cancer, urinary tract calculi, spinocerebellar degeneration, malignant lymphoma, myocardial infarction, and Parkinson's disease in one patient each.

There were five cases in which both urinary uracil and dihydrouracil were high. The cases included one case of dihydropyrimidinuria, one of malignancy, one of severe dehydration, one of renal failure, and one of cerebral infarction.

The mean ± SD urinary dihydrouracil/uracil ratio was 0.36 ± 0.18.

Comparison of the 624 subjects ages 60 years and older

Fig. 2 Distribution of urinary uracil levels in the 1133 subjects. The mean  $\pm$  SD was  $63.81 \pm 66.00 \mu\text{mol/g Cre}$ . The mean  $\pm 1$  SD ranged from 0 to  $129.8 \mu\text{mol/g Cre}$ .

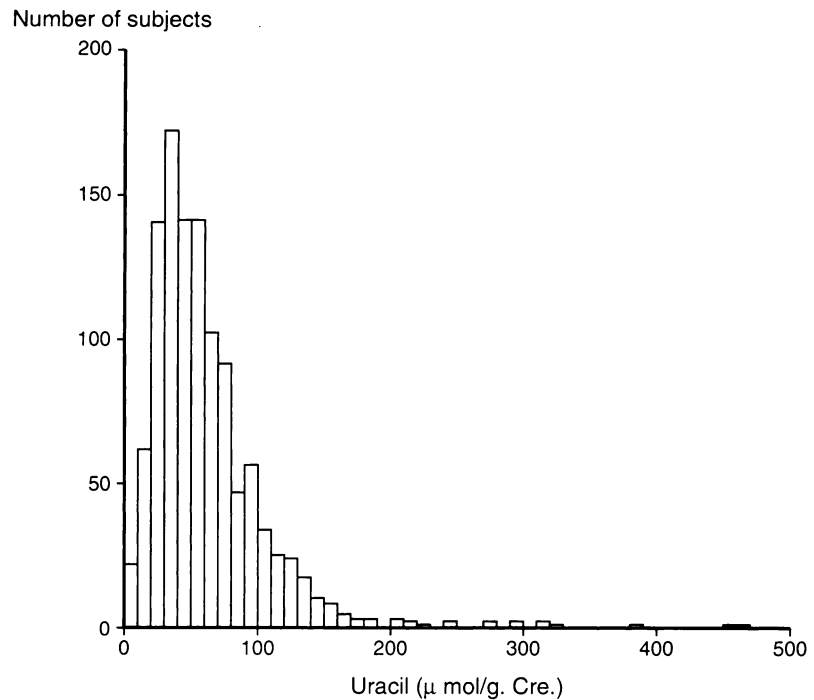


Table 2 Urinary dihydrouracil and uracil concentration in the patients studied<sup>a</sup>

Characteristics	Dihydrouracil ( $\mu\text{mol/g Cre}$ )	Uracil ( $\mu\text{mol/g Cre}$ )
Age		
$\geq 60$ yr ( $n = 624$ )	$23.43 \pm 21.41$	$57.65 \pm 75.85^b$
$< 60$ yr ( $n = 509$ )	$25.37 \pm 46.58$	$73.56 \pm 59.20^b$
Sex		
Male ( $n = 624$ )	$21.34 \pm 41.99^c$	$53.99 \pm 48.28^d$
Female ( $n = 509$ )	$27.03 \pm 24.37^c$	$76.69 \pm 82.02^d$
Diseases		
Healthy subjects ( $n = 167$ )	$23.81 \pm 35.54$	$63.34 \pm 66.03^e$
Hypertension ( $n = 216$ )	$21.37 \pm 14.82$	$53.47 \pm 36.02^e$
Cerebral infarction ( $n = 135$ )	$21.75 \pm 18.49$	$46.24 \pm 36.57^e$
Malignancy ( $n = 51$ )	$25.00 \pm 16.82$	$70.82 \pm 117.12^e$
Liver dysfunction ( $n = 50$ )	$24.22 \pm 17.52$	$62.22 \pm 55.22$
Nephropathy ( $n = 7$ )	$22.32 \pm 18.21$	$58.44 \pm 22.32$

<sup>a</sup> All values are shown as mean  $\pm$  SD (Student *t* test).

<sup>b</sup>  $P = 0.000057$ .

<sup>c</sup>  $P = 0.0043$ .

<sup>d</sup>  $P = 0.034$ .

<sup>e</sup>  $P = 0.021$ .

with the 509 subjects less than 60 years old showed no significant difference in the urinary dihydrouracil level. In contrast, uracil levels were significantly lower in the subjects ages 60 years and older (Table 2). When men and women were compared, both the urinary dihydrouracil and uracil levels were found to be significantly higher in women (Table 2).

Urinary pyrimidine levels were also compared between 216 patients with hypertension, 167 healthy volunteers or subjects undergoing screening, 135 patients with cerebral infarction, 51 patients with malignancy, and 50 patients with liver

dysfunction [GTP ranged from 37 to 358 IU/liter (normal range, 0–35), with a mean  $\pm$  SD of  $90.6 \pm 78.2$ ;  $\gamma$ -GTP ranged from 48 to 913 IU/liter (normal range, 0–47), with a mean  $\pm$  SD of  $183.6 \pm 216.4$ ], and 7 patients with nephropathy [Cre levels ranged from 1.4 to 4.5 mg/dl (normal range, 0.6–1.3), with a mean  $\pm$  SD of  $2.0 \pm 1.0$ ]. No significant differences in the dihydrouracil level were detected between any of these groups, but urinary uracil levels were significantly higher in the patients with malignancy than in the other groups (Table 2). After stratification according to age, sex, and diagnosis, the following reference ranges were determined: 0–59.3  $\mu\text{mol/g Cre}$  for dihydrouracil and 0–129.3  $\mu\text{mol/g Cre}$  for uracil.

**Case Report.** During this study, we detected dihydropyrimidinuria in one asymptomatic adult with suspected dihydropyrimidinase deficiency and assessed the dynamics of pyrimidine metabolism in this individual by performing a uracil loading test. We found an abnormally high urinary dihydrouracil level in a 37-year-old man. He had no abnormal physical or hematological findings, and no abnormalities had been detected by earlier health screening examinations. His dihydrouracil level was extremely high (1854.4  $\mu\text{mol/g Cre}$ ), his uracil level was 240.5  $\mu\text{mol/g Cre}$ , and his dihydrouracil/uracil ratio was 7.7. The man had two children who were healthy, with normal urinary uracil and dihydrouracil levels, and there was no consanguinity in his family.

In this man and three healthy volunteers, an oral loading test was performed with a 10 mg/kg dose of uracil after informed consent was obtained, and the changes in the serum levels of uracil and dihydrouracil were examined. Uracil peaked after about 10 min in the normal subjects but peaked at 67.8  $\mu\text{mol/liter}$  after about 60 min in the man with dihydropyrimidinuria and remained high after 180 min. Eight h after loading, the blood uracil concentration was 11.1  $\mu\text{mol/liter}$  in this man,

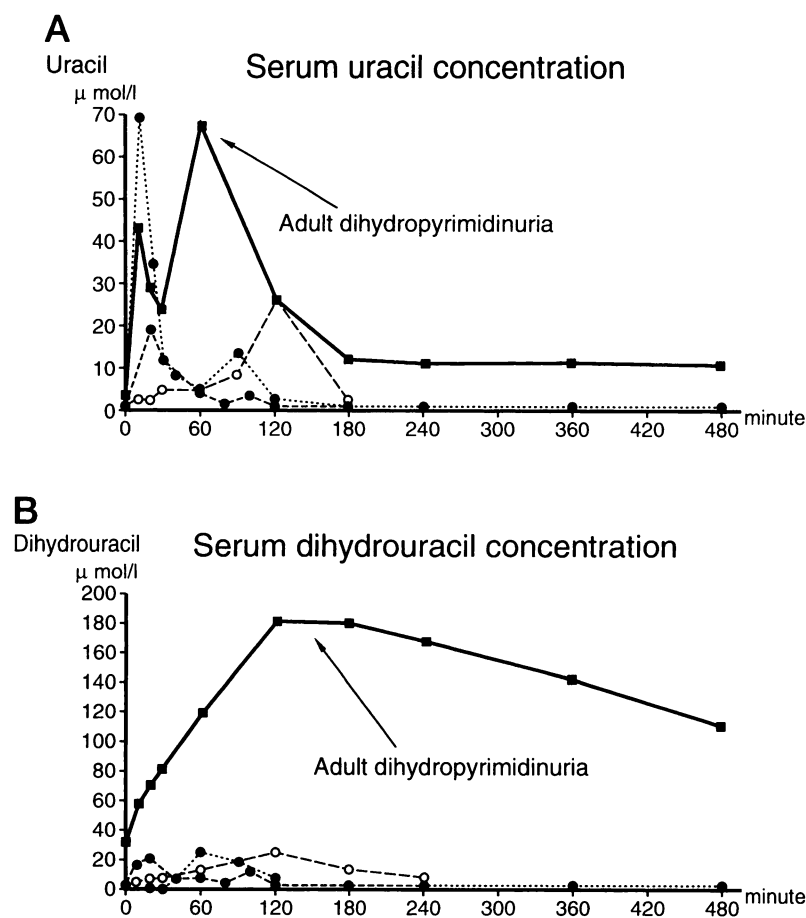


Fig. 3 Uracil loading test in one man with dihydropyrimidinuria and three healthy volunteers. A, persistence of high serum levels of uracil were seen in the man after 180 min (■), compared to the healthy subjects (●, ○). B, very high serum dihydrouracil levels were observed in this man during this loading test.

which was about 17 times higher than in the control subjects. (The uracil loading test showed that, in healthy controls, uracil levels fell to less than  $0.67 \mu\text{mol/liter}$  after 3 h, the  $C_{\text{max}}$  of uracil in the normal subjects was  $38.2 \mu\text{mol/liter}$ , and the  $C_{\text{max}}$  time was 50.0 min (Fig. 3).

In addition, the dihydrouracil level of this man was  $32.5 \mu\text{mol/liter}$  before uracil loading, peaked at  $192.1 \mu\text{mol/liter}$  after about 120 min, and remained high thereafter ( $117.3 \mu\text{mol/liter}$  after 8 h; Fig. 3). In contrast, the dihydrouracil level remained below  $25.7 \mu\text{mol/liter}$  in the healthy controls.

## DISCUSSION

DPD deficiency has been reported outside Japan and has been diagnosed on the basis of peripheral blood DPD activity (4), but there has been little investigation of the changes in urinary pyrimidine levels. Our column-switching chromatography method (8–10) allows measurement of pyrimidines using a small volume of urine (10–50  $\mu\text{l}$ ), thus providing a noninvasive test that is convenient for the doctor and patient.

Our data obtained in 1133 adults did not show any significant age-related differences in urinary dihydrouracil levels, but uracil levels were significantly lower in the subjects ages 60 years and older than in those less than 60 years of age. In addition, urinary uracil levels in the subjects with malignancy were high. Increased

uracil levels in patients with malignancy and in subjects less than 60 years of age may have been attributable to increased nucleic acid metabolism. Both dihydrouracil and uracil levels were significantly higher in women than men. Some researchers say that peripheral DPD activity is higher in women (4). This may indicate increased pyrimidine metabolism with higher urinary dihydrouracil and uracil levels in women.

Twenty of the 1133 subjects had urinary dihydrouracil levels of  $59 \mu\text{mol/g Cre}$  or higher, and 29 had uracil levels of  $130 \mu\text{mol/g Cre}$  or higher. There were five cases in which both urinary dihydrouracil and uracil were high. Both phenomena are seen when there is inhibition of the dihydropyrimidinase enzyme and may be seen when there is advanced cellular necrosis.

Asymptomatic dihydropyrimidinuria was detected in one man with a high urinary dihydrouracil level, and abnormal pyrimidine metabolism was confirmed by the uracil loading test. If such individuals are treated with 5-FU or its derivatives, blood levels will be far higher than in normal adults, and there is a possibility of serious adverse reactions. The urinary pyrimidine levels of this man's parents were within the reference range, and there was no consanguinity, so the pattern of inheritance of dihydropyrimidinuria remains unknown (11). Although it may be possible to screen carriers by analyzing random urine samples, it may be necessary to perform the uracil loading test and

to examine the family members as well. In our present and previous studies, we have detected dihydrophrimidinuria at a rate of approximately 1:2500 in infants and 1:1000 in adults. Thus, this may not be such a rare condition and should be kept in mind when treating patients with 5-FU.

Although the urinary dihydrouracil levels in our infant (7) and adult with dihydropyrimidinuria were about 40-fold higher than those in healthy subjects, problems remain with respect to screening for carriers and partial deficiency. Thus, additional studies of pyrimidine kinetics are needed in patients with urinary dihydrouracil and uracil levels above the reference range.

In conclusion, urinary screening causes little inconvenience to the patient, and performing this test before administering 5-FU may possibly prevent serious adverse effects. However, it is necessary to conduct additional studies on enzyme deficiencies related to pyrimidine metabolism before the value of such testing can be confirmed.

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