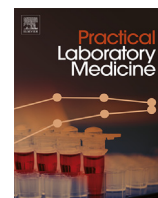




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Distribution of urinary gamma-glutamyltransferase activity in 40- to 74-year-old Japanese women

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ABSTRACT

Urinary gamma-glutamyltransferase (u- γ GT) concentration (U/L) and excretion (urinary creatinine-corrected u- γ GT; u- γ GT/u-Cre, U/g creatinine) are useful markers for kidney disease. However, there is limited information available on u- γ GT and u- γ GT/u-Cre distribution in the elderly Japanese population. In this study, we investigated the distribution of u- γ GT and u- γ GT/u-Cre in 113 Japanese women aged 40–74 years. The u- γ GT was assessed from spot urine samples (collected from 09:00 to 14:00) spectrophotometrically according to the Japan Society of Clinical Chemistry reference measurement procedure using L- γ -glutamyl-3-carboxy-4-nitroanilide as the substrate. The u-Cre was measured enzymatically using creatininase, creatinase, sarcosine oxidase, and peroxidase. None of the participants was diagnosed with any kidney disease. Median u- γ GT and u- γ GT/u-Cre values (central 95% interval values) were 29.7 (5.3–144.0) U/L and 57.9 (32.9–122.7) U/g creatinine, respectively. The distribution of u- γ GT tended to decline with age. There was a statistically significant difference in the u- γ GT value between the 40–59- and 60–74-year-old groups. In contrast, there was no significant difference in the u- γ GT/u-Cre between each age group. The u-Cre level also declined with age. It is suggested that the decline of u- γ GT with aging would be masked by the u-Cre correction.

1. Introduction

Gamma-glutamyltransferase (γ GT) is a membrane enzyme that plays a vital role in glutathione metabolism and shows particularly high activity in the kidney, pancreas, and liver [1,2]. In the kidney, γ GT is strongly expressed on the luminal surface of the proximal tubular epithelial cells [3] and then excreted through the urine. Previous studies have indicated that the urinary γ GT (u- γ GT) concentration and/or excretion, as determined by the urinary creatinine-corrected value of u- γ GT (u- γ GT/u-Cre), can serve as biomarkers for proximal tubular injury given their increased levels in diseases and conditions, such as acute renal injury [4,5], systemic lupus erythematosus [6], heavy metal intoxication [7], drug intoxication [8,9], multiple myeloma [10], and failure of renal transplantation [11].

For practical applications as a biomarker, it is crucial to determine the u- γ GT and u- γ GT/u-Cre distribution in individuals without renal disease as a reference; however, this information is currently limited. In particular, there is limited information available on the u- γ GT and u- γ GT/u-Cre distribution in old Japanese adults. Given that u- γ GT undergoes a physiological change during aging [12] and the urinary Cre concentration differs among age groups [13], it is necessary to establish the u- γ GT and u- γ GT/u-Cre distribution for each age group.

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In Japan, “specific health checkups and specific health guidance” are carried out for all (40–74)-year-old insured individuals and their dependents to improve screening and detection of metabolic syndrome. Therefore, in this study, we used the data available from these “specific health checkups” to determine the distribution of γ GT and u- γ GT/u-Cre in (40–74)-year-old Japanese women. We then compared the u- γ GT concentration and excretion levels in each age group within this cohort.

2. Materials and methods

2.1. Participants

Urine samples were obtained from (40–74)-year-old women ($n = 152$) who received specific health checkups in Choshi, Japan in July and August 2017. Only women were included in the study since the primary participants of the health checkups offered by the local government carries are housewives and elderly individuals.

None of the women was hospitalized or resided in a nursing home at the time of the examination. Individuals were not excluded if they had an illness or disease history, as it is challenging to gather data from only completely physically healthy individuals, especially for more elderly individuals. However, given the likely influence of u- γ GT on kidney disease, women diagnosed with kidney disease were excluded from the study. In addition, 37 people who showed eGFR < 60 mL/min/1.73 m² and/or urinary Alb ≥ 30 mg/g creatinine, which are two diagnostic criteria of chronic kidney disease in Japan [14], were excluded from the analysis. In total, urinary u- γ GT was investigated in 113 (40–74)-year-old females (16 in their 40s, 23 in their 70s).

2.2. Sample and data collection

The urine samples were spot-urine collected from 9:00 to 14:00 and stored at 4 °C in sterilized polypropylene tubes; u- γ GT analysis was carried out on the same day of collection as u- γ GT activity is unstable [15]. Afterward, the urine samples were stored at –30 °C until u-Cre and u-albumin analyses. Collected urine samples were centrifuged at 1500×g for 10 min, and the supernatants were used for the analyses. Physical exam (height, weight, abdominal girth, systolic, and diastolic blood pressure) and biochemical blood parameter values [high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), triglyceride (TG), asparagine aminotransferase (AST), alanine aminotransferase (ALT), serum- γ GT (s- γ GT), hemoglobin A1c (HbA1c; NGSP value), serum-creatinine (s-Cre), estimated glomerular filtration rate (eGFR; estimated by the eGFR equation for Japanese population [16])] were obtained from the Health and Welfare Center of Choshi.

2.3. Analysis of the urinary levels of γ GT, Cre, and albumin

The u- γ GT was measured according to the Japan Society of Clinical Chemistry (JSCC) reference measurement procedure using L- γ -glutamyl-3-carboxy-4-nitroanilide (GluCANA) as the substrate [17]. In brief, 0.15 mL of urine was dispensed into a test tube to which 2 mL of 159 mmol/L glycylglycine buffer (pH 7.90 at 30 °C) was added, and the mixture was incubated in a thermostat chamber at 37 °C for 5 min. Subsequently, 0.5 mL of 31.8 mmol/L GluCANA-glycylglycine buffer was added, and the mixture was further incubated at 37 °C for 1 min. Immediately after incubation, absorbance at 410 nm was measured at 37 °C for 3 min on a Hitachi 7012 model clinical spectrophotometer (Hitachi High-Technologies Corp., Tokyo, Japan). The apparent molar absorption coefficient ($app.\epsilon$) of 5-amino-2-nitrobenzoate (5-ANB), used as the enzyme reaction indicator of our spectrophotometer, was 7525 L·mol⁻¹·cm⁻¹ (37 °C). The γ GT activity was then calculated using the following formula:

$$\gamma\text{GT (U/L)} = \frac{\Delta\text{Abs}/\text{min}}{app.\epsilon} \times \frac{V}{v} \times 10^6$$

where $\Delta\text{Abs}/\text{min}$ is the absorbance change per minute, $app.\epsilon$ is the apparent molar absorption coefficient (L·mol⁻¹·cm⁻¹), V is the total volume of the reaction solution (mL), and v is the sample volume (mL). In this analytical method, the lower and upper limits of quantitation were 2.3 and 800 U/L, respectively. The inter- and intra-assay coefficient of variations (CVs) were 2.15% and 1.71%, respectively.

Urinary creatinine (u-Cre) levels were also measured for the creatinine correction of the u- γ GT by an enzymatic method using creatininase, creatinase, sarcosine oxidase, and peroxidase (L-type WAKO CRE·M, FUJIFILM Wako Pure Chemical, Osaka, Japan) and the clinical autoanalyzer, HITACHI 3100 (Hitachi High-Technologies Corp.). The urine sample was diluted 10-fold with ultrapure water. Controls WAKO-I and –II (FUJIFILM Wako Pure Chemical) were used for γ GT and creatinine measurement quality control. Urinary albumin (u-Alb) levels were also measured by the immune nephelometric method using a polyclonal anti-human albumin antibody (Auto WAKO microalbumin, FUJIFILM Wako Pure Chemical) and the clinical autoanalyzer, HITACHI 3100. Control for Auto WAKO microalbumin (FUJIFILM Wako Pure Chemical) was used for u-Alb measurement quality control.

2.4. Statistical analysis

The normality of each clinical test value was checked by histogram and tested using the Shapiro-Wilk test. Parametric data were indicated as the mean \pm standard deviation, and nonparametric data were indicated as median and interquartile range. Correlations between u- γ GT, u-Cre, u- γ GT/u-Cre, and each medical examination variable were determined by Spearman correlation coefficients

using subject means. Statistical significance was determined by a two-sided Mann-Whitney *U* test. Differences with a *P*-value (*P*) < 0.05 were considered statistically significant. SPSS (version 19, IBM Japan, Tokyo, Japan) was used for statistical analyses.

3. Results

3.1. Distribution of u- γ GT and u- γ GT/u-Cre

The fundamental statistics of physical exams, and blood and urinary biochemical parameter levels for the 113 participants are shown in Table 1. The histograms of the u- γ GT and u- γ GT/u-Cre values for the 113 participants are shown in Fig. 1. Both histograms display a left-skewed distribution. These distributions were indicated as non-parametric by the Shapiro-Wilk test. Therefore, values of u- γ GT and u- γ GT/u-Cre are shown as minimum and maximum, and 2.5, 25, 50 (median), 75, and 97.5 percentiles (Table 2). The u- γ GT and u- γ GT/u-Cre levels ranged from 3.1 to 302.5 U/L and from 8.0 to 168.1 U/g creatinine, respectively, in all the 113 participants.

The distributions of u- γ GT and u- γ GT/u-Cre tended to decline with age. The u- γ GT drastically decreased between the 50s and 60s. There was a statistically significant difference in the u- γ GT value between the (40–59)- and (60–74)-years-old groups according to the Mann-Whitney *U* test (*P* = 0.007). In contrast, the u- γ GT/u-Cre showed a slight decrease between the 60s and 70s, whereas the difference in u- γ GT/u-Cre values between (40–69)- and (70–74)-years-old groups was not significant according to the Mann-Whitney *U* test (*P* = 0.295).

3.2. Correlations between urinary γ GT and medical examination parameters

A statistically significant correlation was observed between u- γ GT and age ($r = -0.236$, *P* = 0.012), height ($r = 0.223$, *P* = 0.018), weight ($r = 0.208$, *P* = 0.027), u-Cre ($r = 0.892$, *P* < 0.001), u-Alb ($r = 0.432$, *P* < 0.001), and u- γ GT/u-Cre ($r = 0.377$, *P* < 0.001). No significant correlations were observed between u- γ GT and abdominal girth, systolic and diastolic blood pressures, HDL-C, LDL-C, TG, AST, ALT, s- γ GT, HbA1c, s-Cre, eGFR, or u-Alb/u-Cre. No significant correlation was observed between u- γ GT/u-Cre and medical examination parameters.

4. Discussion

In this study, we measured the u- γ GT levels in (40–74)-year-old Japanese females (*n* = 113). We also measured the creatinine-corrected value u- γ GT/u-Cre, which is commonly used in clinics since the u- γ GT of spot urine can be affected by the dilution and concentration of urine. A statistically significant positive correlation (*P* < 0.001) was observed between u- γ GT and u-Cre suggesting creatinine correction is reasonable for u- γ GT. Conti et al. reported that correcting any tubular marker to u-Cre is not appropriate; however, u-Cre correction is appropriate for pure glomerulopathy [18]. This study differs from Conti et al. in the background of the

Table 1

Physical exam and biochemical parameter values of the study subjects. Height and blood pressure are shown as mean \pm SD. Others are shown as median (interquartile range).

Variable	n = 113
Physical exam	
Age (y)	66 (58–69)
Height (cm)	153 \pm 6
Weight (kg)	50 (45–57)
Belly girth (cm)	80 (73–85)
Body mass index (kg/m ²)	22 (19–24)
Systolic blood pressure (mmHg)	126 \pm 16
Diastolic blood pressure (mmHg)	72 \pm 10
Blood biochemical parameter	
HDL cholesterol (mmol/L)	1.6 (1.4–1.9)
LDL cholesterol (mmol/L)	3.1 (2.5–3.6)
Triglycerides (mmol/L)	1.0 (0.7–1.5)
AST (U/L)	20 (18–23)
ALT (U/L)	16 (13–19)
γ GT (U/L)	17 (14–23)
HbA1c (NGSP value; %)	5.7 (5.5–6.0)
Creatinine (μ mol/L)	56.6 (52.2–61.0)
eGFR (mL/min/1.73m ²)	72.3 (66.1–79.2)
Urine biochemical parameter	
Albumin (mg/L)	2.6 (0.9–5.2)
Albumin/creatinine (mg/g creatinine)	5.1 (2.1–8.5)
Creatinine (g/L)	0.58 (0.27–0.90)
All subjects	0.85 (0.32–1.44)
40s	0.74 (0.52–1.05)
50s	0.49 (0.24–0.89)
60s	0.56 (0.30–0.72)
70s	

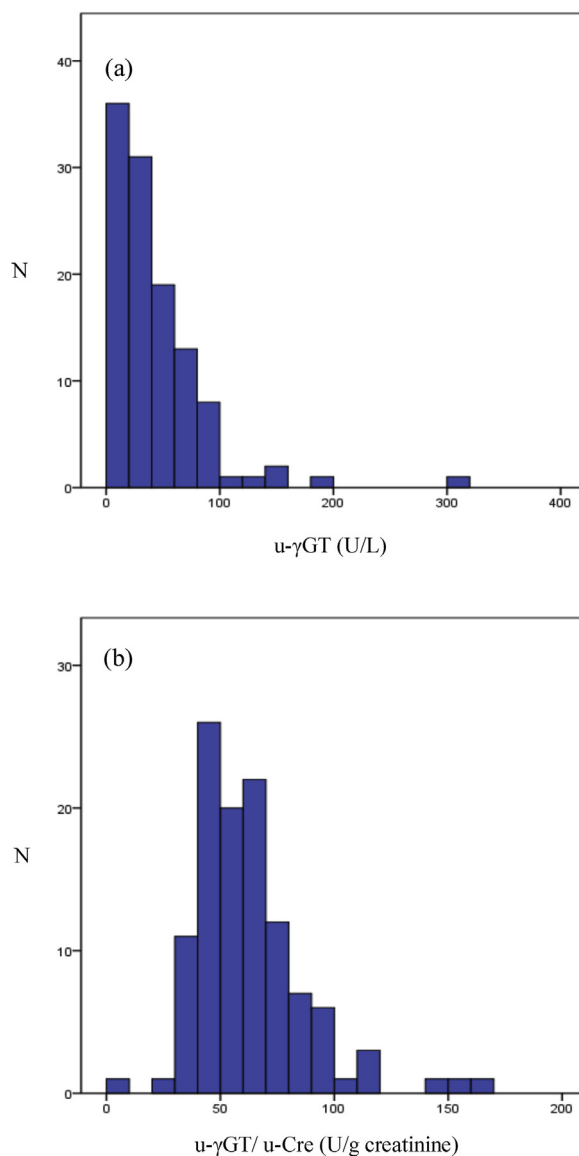


Fig. 1. Histograms of the (a) u- γ GT and (b) u- γ GT/u-Cre levels in the 113 participants.

participants, the type of urine collection, and the analytical method of creatinine. Study participant in the study of Conti et al. is a patient with renal disease on the other hand in our study, participants did not have renal disease. Urine sample in the study of Conti et al. was taken at 24 h urine, while in our study, urine type was spot urine. Analytical method for creatinine in the study of Conti et al. was Jaffe method, but we followed the enzymatic method. Therefore, we cannot compare both the studies directly, and further research is necessary to clarify the relationship between urine concentration and u- γ GT. For the individuals in this study, u- γ GT declined with age. We detected a statistically significant negative correlation between u- γ GT and age ($P = 0.012$), and a statistically significant difference in the u- γ GT value between the (40–59)- and (60–74)-year-old groups ($P = 0.007$). The decline of u- γ GT with age has previously been described [12,19]. During the aging process, there is a decrease in the number of nephrons and a shortening of the renal tubular length [20,21]. Since u- γ GT is of renal tubular epithelial cell origin, this result suggests that the decrease in renal tubular epithelial cells during aging influences u- γ GT. By contrast, no such correlation with age was detected for u- γ GT/Cre. In this study, a statistically significant correlation was observed between u-Cre and age ($r = -0.237$, $P = 0.012$). The negative correlation between u-Cre and age is considered to reflect a decrease in the quantity of muscle with aging [13]. This is considered to reflect the fact that u- γ GT and u-Cre decrease together during aging [12,19,22], and therefore their ratio will cancel out this association.

The u-Cre correction premise is that u-Cre excretion is 1 g/day. However, the quantity of u-Cre excretion changes under the influence of age, gender, race, body mass index, diabetes, and kidney function [22]. The u-Cre correction values in children and elderly women who have low u-Cre levels would be overestimated. If the quantity of u-Cre excretion is 1 g/day and the daily volume of urine of a

Table 2
Urinary γ GT and γ GT/creatinine values of the study subjects.

	All subjects	40s	50s	60s	70s
N	113	16	14	60	23
γ GT (U/L)					
Minimum	3.1	7.9	18.0	3.1	4.3
2.5 percentile	5.3	8.1	18.4	5.6	4.5
25 percentile	17.0	20.1	31.8	14.6	14.9
Median	29.7	45.7	46.9	25.0	27.4
75 percentile	54.2	91.0	61.2	54.2	42.5
97.5 percentile	144.0	262.6	95.0	115.2	73.0
Maximum	302.5	302.5	108.4	143.0	78.2
γ GT/creatinine (U/g creatinine)					
Minimum	8.0	43.3	41.4	8.0	35.0
2.5 percentile	32.9	43.5	42.2	29.6	35.4
25 percentile	45.6	53.5	46.9	44.8	41.0
Median	57.9	60.6	59.1	59.4	51.7
75 percentile	74.9	78.0	70.4	77.2	66.1
97.5 percentile	122.7	139.0	90.3	127.2	98.0
Maximum	168.1	151.7	90.9	168.1	107.7

Japanese woman is 1.2 L/day [23], the u-Cre concentration is estimated as 0.83 g/L. The medians of u-Cre for individuals in their 40s, 50s, 60s, and 70s in this study were 0.85, 0.74, 0.49, and 0.56 g/L, respectively. In this study, the urine samples were collected from 9:00 to 14:00. Concentrations of u-Cre are higher during the morning than in the evening [22,24]. In addition, eating and drinking are limited before specific health checkups. Collectively, this indicates that the average quantity of u-Cre excreted in the urine of elderly persons is < 1 g/day. It is suggested that the u- γ GT/u-Cre values were overestimated in elderly women. Namely, the expected decline of u- γ GT with aging would be masked by the u-Cre correction. A suitable u- γ GT correction method for spot urine in elderly persons is necessary. Recently, Jain [24] has reported a correction formula for u-Cre that is based on age, race/ethnicity, and gender, which may be a useful method. Urinary cystatin C concentration may be a better marker of renal tubular injury in renal-transplant recipients than u- γ GT concentration [18]. For most urinary disease markers, including u- γ GT, urinary dilution and concentration correction are required. Because there are many factors that may cause variations in urinary creatinine levels, it is difficult to evaluate the validity of creatinine correction values. Therefore, it is thought that urinary disease markers, such as urinary cystatin C, that do not require correction for urinary dilution and concentration are more useful.

With regards to physical parameters (height, weight, abdominal girth, systolic and diastolic blood pressure), a statistically significant correlation was observed between u- γ GT and height or weight. Indeed, the number of nephrons has been reported to be proportional to body habitus [25,26], which may explain this correlation.

The u- γ GT correlated with u- γ GT/u-Cre and u-Alb. The correlation between u- γ GT and u- γ GT/u-Cre is quite natural because u- γ GT/u-Cre was calculated from u- γ GT. The correlation of u- γ GT with u-Alb has also been reported in the younger population [19] and is known to fluctuate in response to urinary dilution and concentration.

This study has some limitations. First, the number of subjects ($n = 30$) in the 40–59-year-old age group was small and therefore, may not be good representative of the population from which they were drawn. The difference in the sample size between the (40–59)-year-old group ($n = 30$) and the 60–74-year-old group ($n = 83$) may reduce the statistical power of the Mann-Whitney U test. Second, there were only weak correlations between age and u- γ GT or u-Cre. These observations might cast doubt on the suggestion of this study that creatinine-corrected u- γ GT values are overestimated in elderly women.

In this study, we determined the urinary γ GT distribution in (40–74)-year-old Japanese women receiving specific health checkups, whereby we aimed to provide basic data for the establishment of urinary γ GT reference ranges for each included decade of age. Median u- γ GT tended to decline with age, similar to previous studies [12,19]. For elderly women, the u- γ GT/u-Cre level that is generally used as a disease biomarker may overestimate the amount of u- γ GT excreted.

Ethical considerations

The study plan was approved by the Ethical Committee for Medical and Health Research Involving Human Subjects of Chiba Institute of Science (approval number 28–24) and Department of Sports Science at Juntendo University (approval number 29–57). All the participants provided informed consent for the use of their urine samples and medical examination information. The Health and Welfare Center of Choshi-city (Chiba prefecture, Japan) implemented the organization of the medical examinations and provided permission for the use of the data in this study.

CRedit authorship contribution statement

Akihisa Hata: Investigation, Formal analysis, Data curation, Writing - original draft, Visualization. **Maki Miyauchi:** Investigation, Resources. **Yoshio Suzuki:** Resources, Investigation, Project administration. **Yuki Otomo:** Investigation, Validation. **Noboru Fujitani:** Conceptualization, Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plabm.2020.e00161>.

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Declaration of competing InterestCOI

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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