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Paradoxical effects of thyroid function on glomerular filtration rate estimated from serum creatinine or standardized cystatin C in patients with Japanese Graves' disease

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ABSTRACT

Background: Estimated glomerular filtration rate (eGFR) is clinically valuable for evaluating renal function. Recently, serum cystatin C (sCysC) measurement has been standardized and has demonstrated utility as a novel indicator of renal function. Thyroid hormone is known to affect serum creatinine (sCr) and sCysC, however, the clinical significance of post-treatment renal function evaluation is yet to be completely elucidated. This study examined the effects of thyroid hormones on eGFR by sCr ($eGFR_{Cr}$), and standardized sCysC ($eGFR_{CysC}$) in patients with Japanese Graves' disease (GD).

Methods: Serum samples were obtained from 113 outpatients with GD. Following pharmacotherapy, 41 of the 113 outpatients with GD achieved remission. Renal function was evaluated by $eGFR_{Cr}$ and $eGFR_{CysC}$. Reference method used Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations.

Results: $eGFR_{Cr}$ levels significantly increased whereas $eGFR_{CysC}$ levels significantly decreased with elevated FT3 and FT4 levels in patients with GD. In the remission group, $eGFR_{Cr}$ levels significantly decreased and $eGFR_{CysC}$ levels significantly increased. No significant differences between $eGFR_{Cr}$ and $eGFR_{CysC}$ levels were observed. Furthermore, CKD-EPI equations show a similar trend and $eGFR_{Cr-CysC}$ levels were no significant differences regardless of before and after treatment.

Conclusions: Renal function evaluation by $eGFR_{Cr}$ and $eGFR_{CysC}$ had clinical utility in post-treatment euthyroidism.

1. Introduction

Serum creatinine (sCr) is a well-known biomarker of acute kidney injury and has utility in the clinical evaluation of renal function, however, sCr levels are known to be affected by body muscle mass [1–2]. Recently, serum cystatin C (sCysC) has also been demonstrated as an independent renal function biomarker compared with sCr. Cystatin C (CysC) is a basic 13 kDa protein synthesized in all nucleated cells. sCysC passes almost freely through renal glomeruli and is absorbed by the proximal tubules. Accordingly, more than 95% of filtered sCysC is reabsorbed, with the remaining 5% excreted in urine [3–4]. Therefore, sCysC is less affected by extra-renal factors and considered as a more accurate biomarker of glomerular filtration rate (GFR) than sCr [5–7]. Hyperthyroidism affects sCr and sCysC levels [8–10]. A paradoxical increase in sCysC with decrease of sCr is a consistent finding in hyperthyroidism [11–12]. Nonetheless, the effect of hyperthyroidism on sCr and sCysC levels is not yet completely elucidated. The association between hyperparathyroidism and renal biomarker levels is clinically important, as the prevalence of Graves' disease (GD) is approximately 0.5% in the Japanese population and is the cause of hyperthyroidism in 50–80% [13]. Accordingly, we first aimed to determine the association between estimated GFR (eGFR), calculated by sCr levels ($eGFR_{Cr}$) or standardized sCysC levels ($eGFR_{CysC}$), and thyroid hormone levels in Japanese adult patients with GD and control subjects. Second, we aimed to evaluate changes in $eGFR_{Cr}$

Abbreviations: sCysC, serum cystatin C; sCr, serum creatinine; eGFR, estimated glomerular filtration rate; $eGFR_{Cr}$, estimated glomerular filtration rate calculated by serum creatinine; $eGFR_{CysC}$, estimated glomerular filtration rate calculated by standardized serum cystatin C; $eGFR_{Cr-CysC}$, estimated glomerular filtration rate calculated by serum creatinine and standardized serum cystatin C; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; GD, Graves' disease; RI, reference interval; KDIGO, Kidney Disease Outcome Quality Initiative-Kidney Disease Improving Global Outcomes; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

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and $eGFR_{CysC}$ levels evaluated before and after pharmacotherapy in patients with hyperthyroidism. This study examined the effects of hyperthyroidism on eGFR, $eGFR_{Cr}$, and $eGFR_{CysC}$ according to remission or non-remission status. The effect of thyroid hormone levels [free triiodothyronine (FT3) and free thyroxine (FT4)] on GFR_{Cr} and $eGFR_{CysC}$ was examined in Japanese adult patients with GD.

2. Materials and methods

2.1. Study population

Serum samples were collected from 113 adult patients with GD with untreated or poorly controlled disease (remission group, 41 patients; non-remission group, 72 patients) treated between March 2013 and September 2014 at Chiba University Hospital, Chiba, Japan. Outpatients with GD who met the following inclusion criteria were enrolled: serum thyroid stimulating hormone (TSH) levels <0.1 mIU/L; serum FT3 and/or FT4 levels above reference interval (FT3 > 5.7 and/or FT4 > 19.1 pmol/L); sCr and sCysC levels within reference interval (sCr < 92 μmol/L in males and <70 μmol/L in females, and sCysC < 1.15 mg/L in males and <0.96 mg/L in females). Patients with any disease known to affect renal function were excluded. Hospitalized patients were excluded as sCr may be affected by a loss of body muscle mass. Following pharmacotherapy according to the Japan Thyroid Association guidelines (methimazole and/or propylthiouracil), TSH, FT3, and FT4 levels responded to normal ranges in the remission group (TSH, 0.35–4.94 mIU/L; FT3, 2.6–5.7 pmol/L; and FT4, 9.0–19.1 pmol/L) and did not respond in the non-remission group (TSH < 0.35 mIU/L; FT3 > 5.7 pmol/L; and/or FT4 > 19.1 pmol/L) during the treatment interval. Patients with GD included 24 men and 89 women. Patients younger than 19 years or older than 91 years were excluded according to the guidelines of the Japanese Society of Nephrology. In total, 146 age-matched healthy volunteers were enrolled as control subjects who included 46 men and 100 women without physical or clinical abnormalities upon routine health check. Patients with GD were diagnosed based on symptoms and laboratory examinations, including suppressed TSH levels and increased thyroid hormone levels (FT3 and FT4) and TSH receptor antibodies. Written informed consent was obtained from all patients and healthy control subjects prior to blood sampling. Our study protocol was approved by the ethics committee of Chiba University Graduate School of Medicine.

2.2. Measurements

Serum samples were obtained using vacuum blood collection tubes with coated glass powder to prevent blood coagulation (Kyokuto Seiyaku, Tokyo, Japan). Samples were centrifuged (Hitachi, Tokyo, Japan) at 3000 g for 7 min and then stored at −80 °C until further use. sCr levels were measured using an enzymatic method (CRE LM; Wako, Osaka, Japan), with a reference interval of 54–92 μmol/L for males and 42–70 μmol/L for females, and a coefficient of variation (CV) of 1.0%. The procedure for measuring sCysC was standardized based on international reference material published in 2010 [14]. Standardized sCysC levels were measured by latex turbidimetric immunoassay (N-assay LA cystatin C; Nittobo, Tokyo, Japan), with a reference interval of 0.60–0.98 mg/L for males and 0.49–0.82 mg/L for females, and a 0.9% CV. Standardized sCysC and sCr were measured using a BioMajesty 8040 analyzer (JEOL, Tokyo, Japan) according to the manufacturer's instructions. Serum TSH, FT3, and FT4 levels were measured by chemiluminescent immunoassay using an ARCHITECT i2000SR analyzer (Abotto Japan, Tokyo, Japan) according to the manufacturer's instructions. Reference intervals were as follows: TSH, 0.35–4.94 mIU/L (CV = 1.6%); FT3, 2.6–5.7 pmol/L (CV = 9.8%); and FT4, 9.0–19.1 pmol/L (CV = 9.9%).

2.3. Calculation of the estimated GFR

$eGFR_{Cr}$ and $eGFR_{CysC}$ were calculated using equations reported by the Japanese Society of Nephrology. In the way of reference methods, $eGFR_{Cr}$, $eGFR_{CysC}$, and $eGFR_{Cr-CysC}$ were calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations as follows reported by the Kidney Disease Outcome Quality Initiative-Kidney Disease Improving Global Outcomes (KDIGO) [14–17]:

$eGFR_{Cr} = 194 \times (sCr \times 0.011312)^{-1.094} \times (Age)^{-0.287}$ (×0.739 if female)

$eGFR_{CysC} = (104 \times (\text{standardized sCysC})^{-1.019} \times 0.996^{Age}$ (×0.929 if female)) − 8 $eGFR_{Cr}$ and $eGFR_{CysC}$ are expressed as mL/min/1.73 m^2 of body surface area. sCr is expressed as mg/dL (if μmol/L/88.6) and standardized sCysC as mg/L [14,15].

$eGFR_{Cr}$(CKD-EPI) = 141 × min (sCr × 0.011312/k, 1)^a × max (sCr × 0.011312/k, 1)$^{-1.209}$ × 0.993^{Age} (×1.018 if female) or (×1.159 if black), where k is 0.7 for females and 0.9 for males, a is −0.329 for females and −0.411 for males, min is the minimum of sCr × 0.011312/k or 1, and max is the maximum of sCr × 0.011312/k or 1.

$eGFR_{CysC}$(CKD-EPI) = 133 × min (standardized sCysC/0.8, 1)$^{-0.499}$ × max (standardized sCysC/0.8, 1)$^{-1.328}$ × 0.996^{Age} (×0.932 if female), where min indicates the minimum standardized sCysC/0.8 or 1, and max indicates the maximum standardized sCysC/0.8 or 1.

$eGFR_{Cr\text{-}CysC}$(CKD-EPI) = 135 × min (sCr × 0.011312/k, 1)^a × max (sCr × 0.011312/k, 1)$^{-0.601}$ × min (standardized sCysC/0.8, 1)$^{-0.375}$ × max (standardized sCysC/0.8, 1)$^{-0.711}$ × 0.995^{Age} (×0.969 if female) or (×1.08 if black), where k is 0.7 for females and 0.9 for males, a is −0.248 for females and −0.207 for males, min (sCr × 0.011312/k, 1) indicates the minimum sCr × 0.011312/k or 1, and max (sCr × 0.011312/k, 1) indicates the maximum sCr/k or 1; min (standardized sCysC/0.8, 1) indicates the minimum standardized sCysC/0.8 or 1, and max (standardized sCysC/0.8, 1) indicates the maximum standardized sCysC/0.8 or 1. $eGFR_{Cr}$ and $eGFR_{CysC}$ are expressed as mL/min/1.73 m^2 of body surface area and sCr as mg/dL (if μmol/L/88.6) or standardized sCysC as mg/L [16,17].

2.4. Statistical analyses

Summary statistics were constructed using frequencies and proportions for categorical data and means and standard deviations (SD) for continuous variables. Student's *t*-test was used to compare groups in all experiments. All comparisons were planned, and statistical tests were two-sided. All statistical analyses were performed using StatFlex version 5.0 (ArTeC Inc., Osaka, Japan). Mean and SD were used for descriptive statistics. p values <0.05 were considered statistically significant.

3. Results

3.1. Thyroid hormone levels and renal function in patients with GD compared with control subjects

No significant differences in age or gender were observed between untreated or poorly controlled patients with GD (n = 113) and control subjects (n = 146, Table 1). sCr levels (42 ± 8 μmol/L) were significantly lower ($p < 0.01$), and $eGFR_{Cr}$ levels (141.4 ± 30.0 mL/min/1.73 m^2) were significantly higher ($p < 0.01$) in untreated or poorly controlled patients with GD than in control subjects (58 ± 12 μmol/L and 107.2 ± 13.5 mL/min/1.73 m^2, respectively, Table 1). Standardized sCysC levels (1.06 ± 0.20 mg/L) were significantly higher ($p < 0.01$), and $eGFR_{CysC}$ levels (73.2 ± 13.3 mL/min/1.73 m^2) were significantly lower ($p < 0.01$) in untreated or poorly controlled patients with GD than in control subjects (0.82 ± 0.08 mg/L and 104.9 ± 13.1 mL/min/1.73 m^2, respectively, Table 1). Conversely, significantly higher $eGFR_{Cr}$

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