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ORIGINAL ARTICLE

Measurements of serum cystatin C concentrations underestimate renal dysfunction in pediatric patients with chronic kidney disease

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Abstract
Background In our clinical experience, cystatin C (CysC) concentrations are not as high as expected in patients with chronic kidney disease (CKD) and high-stage renal dysfunction. We therefore investigated whether measurements of serum CysC result in an underestimation of renal dysfunction in pediatric patients with CKD.
Methods Glomerular filtration rate (GFR) was estimated from serum creatinine (Cr) concentration, using the equation Cr-GFR (%) = [0.30 × body length (m)/serum Cr] × 100; and from serum CysC concentration, using the equation Cys-GFR (%) = (0.70/serum CysC) × 100. We investigated the relationship between GFR estimated by these 2 equations. Patients aged 2–12 years were assorted into 5 groups, based on GFR-Cr categories of <12.5, ≥12.5 to <25, ≥25 to <50, ≥50 to <75, and ≥75%, and GFR-CysC/GFR-Cr ratios were compared in these 5 groups.
Results The median GFR-CysC/GFR-Cr ratio in groups of patients with GFR-Cr of <12.5, ≥12.5 to <25, ≥25 to <50, ≥50 to <75, and ≥75% were 2.28, 1.48, 1.22, 1.18 and 0.98, respectively, with statistically significant differences between any two groups ($p < 0.001$).
Conclusion Measurements of serum CysC concentrations lead to underestimation of renal dysfunction in pediatric patients with CKD.

Keywords Serum cystatin C level · Pediatric chronic kidney disease · CKD · Renal dysfunction

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Introduction

Glomerular filtration rate (GFR) reflects kidney function, and is measured by renal clearance techniques. Inulin clearance is the gold standard for evaluating kidney function, but it cannot be measured easily. Therefore, various other methods are used to determine kidney function.

We previously observed a significant positive correlation between serum creatinine (Cr) concentration and body length in children aged 1–12 years, with body length (m) × 0.30 yielding a value similar to the reference serum Cr concentration [1]. An equation for estimated GFR (eGFR) has been used to assess the relationships among body length, glomerular filtration rate (GFR), and serum Cr concentration, using the equation eGFR (ml/min/1.73 m^2) = κ × body length (cm)/serum Cr value (mg/dl) [2], where the constant κ was assumed to be unvaried in children aged 2–12 years. This equation indicates that at constant body length, GFR is in reciprocal proportion to serum Cr concentration. Serum Cr concentration can be determined as body length (m) × 0.30 if GFR is 100%, therefore eGFR (%) = [0.30 × body length (m)/serum Cr] × 100.

We have also found that reference serum cystatin C (CysC) concentrations gradually decrease during the year after birth, with slightly higher concentrations in 1 year olds (0.76 ± 0.10 mg/L) than in children aged ≥2 years (0.70 ± 0.09 mg/L), and serum CysC concentrations are

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relatively constant in children aged ≥2 years [3]. The reciprocal of CysC concentration may therefore correlate with GFR as well or better than the reciprocal of serum Cr [4]. Thus, eGFR may be derived from serum CysC concentration using the equation eGFR (%) = (0.70/serum CysC) × 100. In our clinical experience, however, CysC levels are not as high as expected in chronic kidney disease (CKD) patients with high-stage renal dysfunction. We therefore investigated whether measurements of serum CysC concentrations result in an underestimation of renal dysfunction in pediatric CKD patients.

Materials and methods

We included a total of 199 children (114 males and 85 females), aged 2–12 years, who had been admitted to or attended the outpatient clinic of Aichi Children's Health and Medical Center between December 2003 and February 2008 for CKD, but had not undergone dialysis or renal transplantation.

Patients from whom consent was not received for inclusion of specimens in a clinical report were excluded. Data on serum Cr values, serum CysC values, and body length measured in daily laboratory tests were reviewed.

Serum Cr concentrations were determined by an enzymatic method, using a Hitachi 7170S automated analyzer (Hitachi High-Technologies Corp.) with Accuras Auto antibody (Shino-Test Corp.). The coefficients of inter- and intra-assay variance were satisfactory (1.31 and 1.80%, respectively).

Serum CysC concentrations were also determined using the Hitachi 7170S automated analyzer and a latex agglutination turbidimetric method (Mitsubishi Chemical Medience Corp.). The coefficients of inter- and intra-assay variance were satisfactory (1.14 and 1.25%, respectively).

We utilized two equations for eGFR [1–4]. In children aged 1–12 years, body length (m) × 0.30 yielded a value similar to the reference serum Cr concentration measured enzymatically [1]. Since the reciprocal of serum Cr correlated with GFR [2, 5, 6], we utilized an equation for eGFR derived from serum Cr

$$\text{Cr - GFR}\ (\%) = [0.30 \times \text{body length (m)}/\text{serum Cr}] \times 100.$$

We also found that normal children aged ≥2 years have relatively constant serum CysC concentrations (0.70 ± 0.09 mg/L) [3]. Since the reciprocal of CysC correlated with GFR [4], we utilized an equation for eGFR derived from serum CysC

$$\text{Cys - GFR}\ (\%) = (0.70/\ \text{serum CysC}) \times 100.$$

The relationship between values obtained from these two equations was plotted by scattergram. Patients were assorted into five CKD stage groups by Cr-GFR, i.e., Cr-GFR <12.5, ≥12.5 to <25, ≥25 to <50, ≥50 to <75, and ≥75%, thought to be approximately equal to an international CKD stage classification, and the GFR-CysC/GFR-Cr ratio was compared among these 5 groups.

Spearman's rank correlation and Mann–Whitney's U test were used for statistical comparisons, and $p < 0.01$ was regarded as statistically significant.

Results

The demographic and clinical characteristics, including any underlying illnesses, of the patients are shown in Table 1. The correlation between GFR-Cr and GFR-CysC in all subjects was compared with a line with a slope of 1.0 that passed through the origin (Fig. 1). In CKD patients with high-stage renal dysfunction, the GFR-CysC levels were generally higher than this line. We therefore compared GFR-CysC/GFR-Cr ratios in five groups of patients at different CKD stages, as assessed by GFR-Cr percentages (Table 2). We found that the median GFR-CysC/GFR-Cr in pediatric patients with GFR-Cr <12.5, ≥12.5 to <25, ≥25 to 50, ≥50 to <75, and ≥75% were 2.28, 1.48, 1.22, 1.18 and 0.98, respectively, with significant differences between any two groups ($p < 0.001$). The

Table 1 Patient characteristics

	Total (n = 199)
Male, n (%)	114 (60.0)
Age (years), median (range)	7 (2–12)
Underlying illness, n (%)	
Vesicoureteral reflux	27 (13.6)
Renal hypoplasia/dysplasia	23 (11.6)
Hydronephrosis	17 (8.5)
Minimal change nephritic syndrome	16 (8.0)
Nephritis	14 (7.0)
IgA nephropathy	11 (5.5)
Neurogenic bladder	9 (4.5)
Reflux nephropathy	9 (4.5)
Megaureter	8 (4.0)
Focal segmental glomerulosclerosis	7 (3.5)
Alport's syndrome	6 (3.0)
Hematuria syndrome	4 (2.0)
Henoch–Schönlein purpura nephritis	4 (2.0)
Autosomal recessive polycystic kidney disease	3 (1.5)
Branchio-oto-renal syndrome	3 (1.5)
Membranoproliferative glomerulonephritis	3 (1.5)
Membranous nephropathy	2 (1.0)
Nephronophthisis	2 (1.0)
Others	31 (15.6)

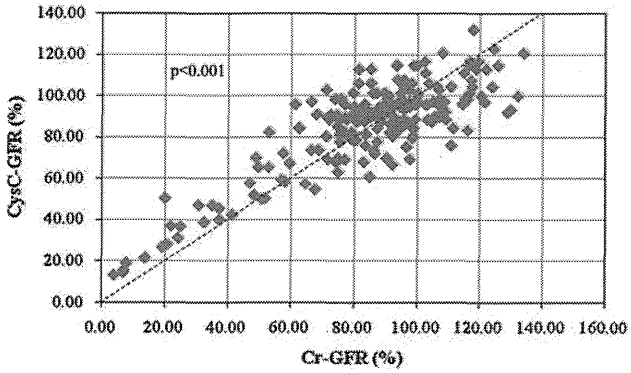

Fig. 1 Correlation between GFR-Cr and GFR-CysC in all subjects. The *dashed line* represents a line with a gradient of 1.0 passing through the origin

Table 2 GFR-CysC/GFR-Cr in five CKD stage groups by GFR-Cr categories

GFR-Cr	*n*	Median (GFR-CysC/GFR-Cr)
<12.5%	4	2.28
≥12.5 and <25%	7	1.48
≥25 and <50%	10	1.22
≥50 and <75%	28	1.18
≥75%	150	0.98

Significant difference between any two groups ($p < 0.001$)

relationships between GFR-Cr strata and GFR-CysC/GFR-Cr are shown in box-and-whisker plots in Fig. 2. The boxes are drawn around the quartile values, and the whiskers extend from each quartile to the smallest and largest observed values. The GFR-CysC/GFR-Cr ratio exceeded 1.0 more often in CKD patients with high-stage renal dysfunction.

Discussion

True measurements of GFR using the inulin clearance method are impractical as a regular monitoring tool. This method is complicated, requiring timed urine collections and frequent blood samplings. It is difficult to obtain timed urine collections from young children because of the physiological immaturity of their bladder function, and this method is even more difficult in children with bladder dysfunction. Therefore, easy, reproducible and precise surrogate methods are needed to measure GFR.

It is necessary to set standard serum Cr and CysC measurements for the medical care of pediatric CKD patients. We previously reported a significant positive correlation between serum Cr concentration and body length in children aged 1–12 years, showing that body

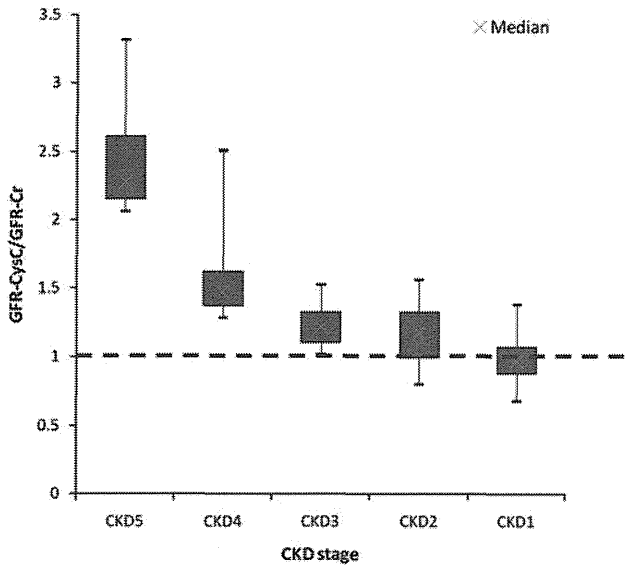

Fig. 2 Relationships between GFR-Cr strata and GFR-CysC/GFR-Cr. *Box-and-whisker* plots of the relationships between GFR-Cr strata and GFR-CysC/GFR-Cr, with significant differences between any two groups ($p < 0.001$). The *boxes* are drawn around the quartile values, and the *whiskers* extend from each quartile to the smallest and largest observed values

length (m) × 0.30 yielded a value similar to the reference serum Cr [1]. In addition, we reported that reference serum CysC concentrations gradually decrease during the year after birth, with slightly higher concentrations in 1 year old children (0.76 ± 0.10 mg/L) than in children aged ≥2 years (0.70 ± 0.09 mg/L) [3].

Although the correlation between reciprocal serum CysC concentration and GFR was reported equivalent to the correlation between serum Cr and GFR, we observed clinically that CysC concentrations were not as high as expected in CKD patients with high-stage renal dysfunction. We therefore determined whether measurements of serum CysC concentrations underestimate renal dysfunction in pediatric CKD patients.

Since the reciprocal of serum Cr has been found to correlate with GFR [2, 5, 6], we defined Cr-GFR (%) as [0.30 × body length (m)/serum Cr] × 100 in children aged 1–12 years. If we assume that the reciprocal of CysC is correlated with GFR, we could define Cys-GFR (%) as (0.70/serum CysC) × 100 at the age of 2 years or over. We compared these two estimated GFR equations by scattergram and by examining the GFR-CysC/GFR-Cr ratio in five groups of patients with CKD stages defined by GFR-Cr. We found that the GFR-CysC/GFR-Cr ratio exceeded 1.0 more often in CKD patients with high-stage renal dysfunction and that there were significant differences between any two groups. If we assume a simple reciprocal relationship between CysC and GFR, serum CysC concentrations lead to underestimations of renal

dysfunction compared with serum Cr in pediatric patients with CKD. At present, the reasons that serum CysC concentrations levels are not as high as expected in CKD patients with high-stage renal dysfunction are unclear. It has been reported that elevation of the serum CysC level slowed down for high-stage adult CKD patients. The existence of non-renal clearance of CysC is indicated and the magnitude is about 20 ml/min/1.73 m^2 in humans [7], which may explain the results in this study.

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SHORT COMMUNICATION

Is the new Schwartz equation derived from serum creatinine and body length suitable for evaluation of renal function in Japanese children?

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Abstract The present study was performed to determine whether the new Schwartz "bedside" equation can be used to estimate the glomerular filtration rate (GFR) in Japanese children as there are differences in renal function and muscle mass between Japanese and American individuals. It is also important to determine whether one common equation can be used in children from 1 to 16 years old, including the period of adolescence. Blood samples were collected from a total of 1,074 healthy children (466 males and 608 females) between 1 and 16 years old. The estimated GFR (eGFR) derived by the new Schwartz bedside formula [eGFR (in milliliters per minute per 1.73 m^2)=0.413×body length (in centimeters)/serum Cr value (in milligrams per deciliter)] was calculated in all subjects, and the relationship between age and eGFR was analyzed. The eGFR decreased gradually with age, and the decrease was more marked in males than females, mainly in adolescence. Weak negative but significant correlations were observed in 466 males and 608 females. The median of the eGFR value showed a gradual significant decrease with age. Conclusion: A common coefficient cannot be used in children between 1 and 16 years old, including the period of adolescence, with the Schwartz type formula, and the new Schwartz bedside formula cannot be used when we estimated GFR in Japanese children. It is necessary to establish an eGFR equation specifically for Japanese children.

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Keywords Reference serum creatinine level · Japanese children · Enzymatic method · New Schwartz formula · eGFR

Introduction

Schwartz et al. expressed the relations between body length, glomerular filtration rate (GFR), and serum Cr level as estimated GFR [eGFR (in milliliters per minute per 1.73 m^2)=κ×body length (in centimeters)/serum Cr value (in milligrams per deciliters)] [2]. The coefficient κ is 0.33 in preterm infants under 1 year old, 0.45 in full-term infants under 1 year old, 0.55 in children 2–12 years old, and 0.55 and 0.70 in females and males over 12 years old, respectively [2–5]. This formula is clinically useful as it allows estimation of the patient's GFR from body length and serum Cr level. This equation utilizes the Jaffe method to measure Cr. However, enzymatic methods have recently been used to measure Cr, making the above formula no longer applicable. In 2009, the updated Schwartz formula, the so-called bedside version, was reported as follows: eGFR (in milliliters per minute per 1.73 m^2)=0.413×body length (in centimeters)/serum Cr value (in milligrams per deciliter) by enzymatic Cr determination in children 1–16 years old [6].

We have previously reported reference serum creatinine levels determined by an enzymatic method in Japanese children according to sex and age [7]. The present study was performed to determine whether the new Schwartz "bedside" equation can be used to estimate the GFR in Japanese children as there are differences in renal function and muscle mass between Japanese and American individuals. It is also important to determine whether one common

equation can be used in children from 1 to 16 years old, including the period of adolescence.

Materials and methods

Blood samples were collected from a total of 1,151 children (517 males, 634 females) between 1 month and 18 years old presenting at the facilities of the members for the Committee of Measures for Pediatric Chronic Kidney Disease (CKD) and Tokyo Health Service Association between 2008 and 2009 without renal, urogenital, infectious, inflammatory, muscular, cardiovascular, liver, and pancreas diseases and not being hypertensive, dehydrated, or pregnant [7]. The study was approved by the local ethics boards of all participating institutions, and written informed consent was obtained from the parents of all subjects. Data from children under 1 or over 16 years old were excluded, and the remaining data from 1,074 children (466 males, 608 females) between the ages of 1 and 16 years were used in this study.

Subjects were divided into the following groups based on age. The eGFR derived by the new Schwartz formula [eGFR (in milliliters per minute per 1.73 m^2)=0.413×body length (in centimeters)/serum Cr value (in milligrams per deciliter)] was calculated in all subjects and the median, 2.5 percentile, and 97.5 percentile values of the eGFR in each age and sex. In all subjects, the relationship between age and eGFR was determined by linear regression analysis.

Serum samples were stored at −70 °C until serum Cr was measured at SRL, Inc. (Tokyo, Japan). The serum level of Cr was determined by an enzymatic method using a Bio Majesty automated analyzer (JCA-BM8060; JEOL Ltd., Tokyo, Japan) with Pureauto S CRE-L (Sekisui Medical Co., Ltd., Tokyo, Japan). The coefficient of variation was satisfactory (2.08 %).

All analyses were conducted using Microsoft Excel 2007 (Microsoft, Redmond, WA) and the statistical software package JMP 8 (SAS Institute Inc, Cary, NC). We conducted linear regression analysis to determine whether the new Schwartz formula can be used to evaluate the renal function in Japanese children. We used Wilcoxon's analysis to compare differences in the reference eGFR values between the ages. In all analyses, $P<0.01$ was taken to indicate statistical significance.

Results

We examined the correlations between eGFR derived by new Schwartz formula and age in males and females (Fig. 1). These scattergrams showed that eGFR decreased gradually with age, and the decrease was more marked in males than females mainly in adolescence. Weak correlations were

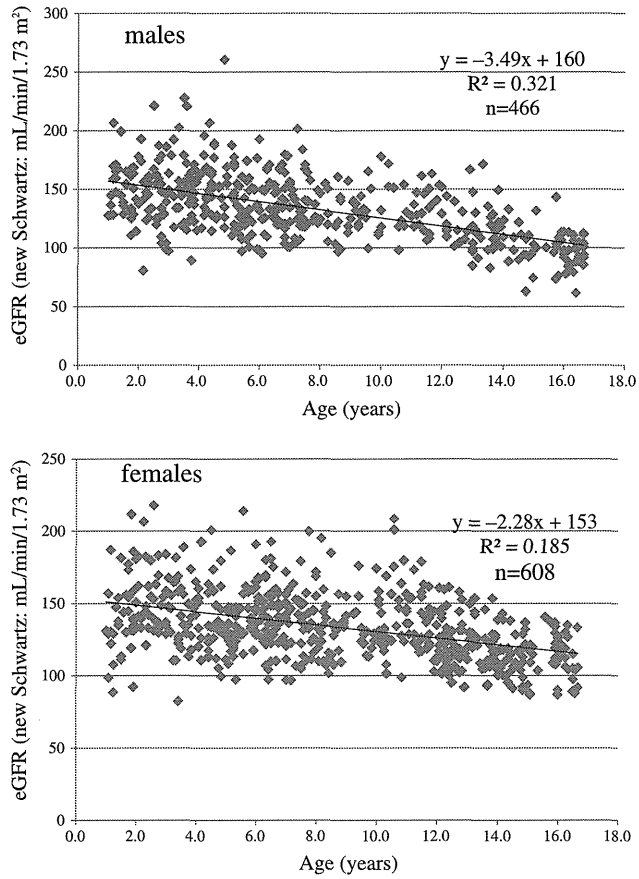

Fig. 1 Correlation between eGFR derived by the new Schwartz bedside formula and age in all male and female subjects, respectively. The scattergram shows that eGFR decreased gradually with age showing a weak but significant negative correlation

observed in 466 males and 608 females, and the correlation coefficients were 0.567 and 0.430 (Fig. 1, $P<0.001$), respectively.

We reviewed the median, 2.5 percentile, and 97.5 percentile of the eGFR value in each age group between 1 and 16 years old in males and females, respectively (Table 1). The median of the eGFR value decreased gradually with age, i.e., 130–150 mL/min/1.73 m^2 between 1 and 11 years old and 95.9 mL/min/1.73 m^2 in males and 112.3 mL/min/1.73 m^2 in females at 16 years old, respectively ($P<0.001$).

Discussion

GFR is used to assess kidney function and is measured by renal clearance. Inulin clearance is the gold standard for evaluation of kidney function but cannot be measured easily. Therefore, various methods have been used to determine GFR. The eGFR (in milliliters per minute per 1.73 m^2)=κ× body length (in centimeters)/serum Cr value (in milligrams per deciliter) by the Jaffe method devised by Schwartz has been used clinically [2]. Recently, however, enzymatic

Table 1 The median, 2.5 percentile, and 97.5 percentile of the eGFR value in each age group between 1 and 16 years old in males and females, respectively

Age (years old)	*n*	2.5 %	50 %	97.5 %
Males				
1	33	113.3	147.5	200.7
2	40	98.0	146.2	193.4
3	48	100.2	148.7	217.6
4	43	116.9	149.6	206.0
5	47	99.2	134.8	177.2
6	43	98.2	134.5	179.7
7	38	103.6	131.4	185.0
8	18	97.8	123.4	159.1
9	18	104.5	130.6	159.0
10	12	103.5	131.3	176.0
11	19	106.9	133.8	162.3
12	15	102.4	116.0	161.4
13	30	84.3	112.5	155.4
14	17	73.1	98.0	129.1
15	15	73.8	103.0	139.2
16	30	73.3	95.9	113.2
Females				
1	36	91.8	136.4	190.1
2	33	128.1	148.7	208.8
3	40	109.8	140.8	184.2
4	38	104.9	136.2	193.4
5	49	108.8	132.7	181.7
6	58	105.4	140.3	186.8
7	47	98.1	137.6	178.1
8	38	106.8	132.7	185.9
9	17	113.5	129.2	167.8
10	32	100.9	132.4	202.7
11	39	110.0	136.9	173.6
12	54	96.6	119.5	160.4
13	38	93.7	121.5	153.5
14	40	91.3	112.3	139.1
15	22	87.3	117.7	138.9
16	27	87.5	112.3	133.9

methods have been used to measure Cr rather than the Jaffe method, so it is not possible to use the formula in this form. Therefore, it was necessary to reevaluate the value of the coefficient κ in the formula. Recently, Zappitelli et al. revised the Schwartz formula relating eGFR to serum creatinine level determined enzymatically and reported that the κ value in the Schwartz equation decreased from 0.55 to 0.47 for children and adolescent girls [8]. Schwartz et al. reported the updated formula, the so-called bedside version, as eGFR=0.413× body length (in centimeters)/serum Cr value (in milligrams per deciliter) by the enzymatic method showing a 25 % reduction in κ value from the previous value of 0.55 generated from Jaffe-based serum Cr measurements [6]. The work was defined from a population of American children with chronic kidney disease, enriched with obstructive uropathy. They concluded that the formula can be used regardless of age or gender in children 1–16 years old. However, the work has been misread and misused to assess eGFR in healthy children.

The present study was performed to determine whether the new Schwartz bedside equation can be used for the evaluation of renal function in Japanese children. Previously, we reported reference serum creatinine levels determined by an enzymatic method in Japanese children according to sex and age [7]. The eGFR derived by the new Schwartz formula [eGFR (in milliliters per minute per 1.73 m^2)= 0.413×body length (in centimeters)/serum Cr value (in milligrams per deciliter)] was calculated for our 1,074 subjects between the ages of 1 and 16 years old and the median, 2.5 percentile, and 97.5 percentile values of the eGFR in each age and sex. The median of the eGFR value showed a gradual significant decrease with age. In addition, the relationship between age and eGFR was determined by linear regression analysis, and weak but significant negative correlations were observed in both male and female subjects. It seems to be a large problem that the ranges of the reference value of boys over 12 years and girls over 14 years old overlap a range of CKD stage 2. In healthy children, normal serum creatinine values are sufficient to define normal kidney function.

Brodehl et al. reported that GFRs derived from inulin clearance approached adult levels within 2 years and were approximately constant between 3 and 15 years old, showing values of 111.2 and 117.2 mL/min/1.73 m^2 at 3–4 years and 13–15 years old, respectively [1]. Our results indicating that eGFR value derived by the new bedside Schwartz formula decreased gradually with age suggest that this formula should not be used for estimating the GFR of Japanese children, at least in those with normal renal function. Weak points of our study are that our materials were healthy not chronic kidney disease children and that they were not actually measured with GFR. In addition, we entrusted the judgment of each coauthor whether each case met our exclusion criteria. We will go ahead through the study of the inulin clearance for patients with Japanese pediatric CKD and intend to review new Schwartz formula in this study.

Schwartz et al. expressed the relationship between body length, GFR, and serum Cr level as eGFR (in milliliters per minute per 1.73 m^2)=κ×body length (in centimeters)/serum Cr value (in milligrams per deciliter) [2], and in this old Schwartz formula, the coefficient κ is 0.55 in children 2–12 years old and 0.70 in males over 12 years old [2, 4]. The new Schwartz formula has an inherent problem with using the same coefficient between the ages of 1 and 16 years old. In addition,

we assume that renal function and muscle mass show ethnic differences.

While indeed it is inappropriate to use the new Schwartz bedside formula in normal Japanese children, it may be inappropriate in all normal children of any ancestry, ethnicity, or national origin. We must realize that it was not defined for these populations of normal children.

In conclusion, the common coefficient cannot be used between 1 and 16 years old, including the adolescent period, in the Schwartz type formula, and the new Schwartz bedside formula cannot be used when we estimated GFR in Japanese children. It is necessary to establish a specialized estimated GFR equation for use in Japanese children and to review new Schwartz formula for patients with pediatric CKD.

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ORIGINAL ARTICLE

Establishment of a normal reference value for serum β2 microglobulin in Japanese children: reevaluation of its clinical usefulness

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Abstract
Objective Serum β2 microglobulin (β2MG) is considered to be a marker of renal function, which is independently associated with age. However, only a few studies have reported the reference values for β2MG in children thus far, particularly in young children. In this study, we evaluated the distribution of serum β2MG values in healthy Japanese children and assessed its clinical usefulness.
Method The normal reference value of serum β2MG was assessed in serum samples from 1131 normal Japanese children (504 boys and 627 girls; age 0–17 years). To test the validity of the reference value, serum samples from children with various kidney diseases were also examined retrospectively.
Results The mean values for β2MG were significantly negatively correlated with age ($r = -0.47$, $P < 0.001$). No significant difference was observed between the values of boys and girls in any age group. The established β2MG reference range covered 99.7 % of patients with decreased kidney function below 75 % based on their serum creatinine (Cr) value and body length.
Conclusion The newly established β2MG reference value in children can be used to detect kidney impairment in children. Serum β2MG in combination with serum Cr used as markers for predicting glomerular function can provide an accurate detection of kidney dysfunction in children.

Keywords β2 microglobulin · Body mass · Children · Chronic kidney disease · Kidney function · Reference value

Introduction

The worldwide increase in the number of patients with chronic kidney disease (CKD) is being recognized as a global public health problem. CKD is not only a cause of end-stage renal disease (ESRD) during childhood but also a key cause of CKD and ESRD in adults. Therefore, the early detection of impaired glomerular function in children, facilitated by routine examinations of kidney function, is essential to inhibit the progression of CKD and reduce the incidence of ESRD. However, this assessment is limited by the lack of markers for impaired kidney function in children. In addition, there are few studies that have established race-based reference values for children.

A multicenter study was recently conducted to establish normal reference values for serum creatinine (Cr), beta 2 microglobulin (β2MG), and cystatin C levels in Japanese children, and a normal serum Cr reference value was established for Japanese children by using an enzymatic detection method [1]. There is a significant correlation between the serum Cr concentration and body length (BL), expressed as BL (m) × 0.30 for children aged 1–12 years, providing a simple formula convenient for estimating glomerular function. A polynomial equation that can predict serum Cr values in children of all ages was also established [1]. Serum Cr is the most widely used marker

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for predicting kidney function. The newly established Cr value for Japanese children will further improve the diagnostic accuracy for detecting reduced renal function. However, Cr concentrations are insensitive to mild reduction in the glomerular filtration rate (GFR). In addition, the age and muscle mass dependencies of serum Cr complicate GFR assessment in children; physicians, particularly if they are not nephrologists or pediatricians, often do not take these complications into account [2, 3]. Therefore, additional markers independent of age and sex are preferable to aid the screening of renal function.

β2MG is a well-established marker that is independent of muscle mass and age; therefore, it has better diagnostic sensitivity than serum Cr for the detection of impaired GFR in growing children and children associated with severe loss of body mass [4, 5]. The production of β2MG, however, is known to increase during infection, inflammatory processes, proliferative syndromes, autoimmune diseases, and malignancies [6], which may affect the evaluation of glomerular function in children. Therefore, it is necessary to establish an accurate range of β2MG in healthy children, which can be used as an accurate diagnostic marker of renal dysfunction in children.

Despite the clinical importance of evaluating the renal function independent of age, sex, and race, there are few studies on normal β2MG reference values in children. Therefore, this large-scale study was performed to evaluate the normal reference values of β2MG in healthy Japanese children.

Materials and methods

Collection of blood samples (multicenter study)

Blood samples were collected from a total of 1151 children (517 boys and 634 girls) between the ages of 1 month and 18 years who presented at the member facilities of the Committee of Measures for Pediatric CKD and the Tokyo Health Service Association between 2008 and 2009 [1]. The study was approved by the local ethics boards, and written consent was obtained from the parents of all subjects. Data lacking β2MG values were deleted, and the remaining data from 1131 healthy children (504 boys and 627 girls) with ages between 1 month and 17 years (mean overall age, 7.7 $\pm$ 4.7 years; mean age of boys, 7.0 $\pm$ 4.8 years; mean age of girls, 8.4 $\pm$ 4.6 years) were used in this study. Children with kidney diseases, urogenital diseases, infectious diseases, inflammatory diseases, dehydration, muscular diseases, anomaly syndrome, malignancies, cardiovascular diseases, and liver or pancreas diseases were excluded from this study.

Measurement of B2MG

The serum samples were stored at -70 °C until further measurements were performed at SRL Inc. (Tokyo, Japan). The serum concentrations of both β2MG and Cr were determined by a latex agglutination immunoassay and an enzymatic method, respectively, by using a Bio Majesty automated analyzer (JCA-BM8060; JEOL Ltd, Tokyo, Japan).

Test validity of reference value

The archival serum β2MG and Cr data collected from patients with various kidney diseases hospitalized between 2004 and January 2010 for routine examinations for clinical management were used to test the validity of the established β2MG reference values. The collected data included 345 serum samples from 21 children with various kidney diseases, including hypoplastic or dysplastic kidney ($n = 8$), kidney injury during the neonatal period ($n = 3$), reflux nephropathy ($n = 1$), post-hemolytic uremic syndrome ($n = 1$), focal segmental glomerulosclerosis (FSGS) ($n = 4$), congenital nephrotic syndrome ($n = 1$), IgA nephropathy ($n = 1$), drug-induced renal dysfunction ($n = 1$), and mitochondrial disease ($n = 1$). The patients were aged 0.1–13.6 years (mean 6.0 $\pm$ 4.8 years) at the time of diagnosis, and all developed decreased GFR during their disease course. Samples were collected when the patients were 0.6–16.9 years of age (mean 8.3 $\pm$ 5.3 years). The mean observation period was 3.1 $\pm$ 2.6 years. The male-to-female ratio was 14:7. All samples were confirmed to be C-reactive protein-negative to exclude the possible effect of inflammation on β2MG values. Medical records for the BL and body weight taken during blood tests were also collected. All patients gave their informed consent at the beginning of treatment for the use of the data in addition to that required for diagnostic purposes, i.e., for research purposes.

Individual serum Cr values and the reference value calculated by the recently established polynomial equation formula were used to evaluate the kidney dysfunction, as follows [1]:

$$\text{For boys: } y = -1.259x^5 + 7.815x^4 - 18.57x^3 + 21.39x^2 - 11.71x + 2.628$$

$$\text{For girls: } y = -4.536x^5 + 27.16x^4 - 63.47x^3 + 72.43x^2 - 40.06x + 8.778,$$

where y is the reference serum creatinine (mg/dl) and x is body length (m).

Thus, kidney function was defined as [patient Cr/reference Cr (y) $\times$ 100 (%)].

Statistical analysis

The statistical analysis was performed with the GraphPad Prism software package (Ver. 5.0; GraphPad Software, San Diego, CA). The reference cohort with β2MG and Cr was subdivided into separate age groups for girls and boys. The differences between the groups were tested with the Kruskal-Wallis nonparametric analysis of variance (ANOVA), Mann-Whitney *U* test, or chi-square analyses as appropriate. The relationship between age and serum β2MG concentration was determined by both linear and polynomial regression analyses. The data are expressed as the mean ± standard deviation (SD) or 95 % confidence interval (CI). Associations between age, BL, serum Cr, and kidney function (%) were assessed with correlation coefficients according to Pearson (*r*). $P < 0.05$ was defined as statistically significant in all analyses.

Results

β2MG reference values in Japanese children

The characteristics of healthy children were as follows: the mean age was 7.8 ± 4.7 years (95 % CI 7.5–8.1 years) with a range of 0.1–16.7 years and a median of 6.9 years. The mean BL was 1.21 ± 0.30 m (range 0.54–1.85 m). There were 64 children who were taking cold medicine or antiallergic agents, though no one had fever or any other symptoms of inflammation. The median, 2.5 percentile, and 97.5 percentile serum β2MG reference values in each subgroup of age are summarized in Table 1. Combining these values as a single cohort yielded a mean serum β2MG concentration of 1.45 ± 0.3 mg/l (95 % CI 1.43–1.47 mg/l). There were no differences in β2MG concentrations between boys and girls of any age group; however, the β2MG data varied widely, particularly in younger subjects (Table 1). It appears that there was a significant change in the value of the upper limit (97.5th percentile) between children aged between 1 and 2 years (Table 1).

Scattergrams show the age-dependent distribution of serum β2MG concentrations (Fig. 1) in which the serum β2MG concentration gradually decreases with age. There is a significant negative correlation among the serum β2MG concentration, age, and BL (both $r = -0.47$, $P < 0.0001$), and the regression equations were $y = -0.0341x + 1.72$ and $y = -0.0055x + 2.12$, respectively (Fig. 1a, b). The relationships between serum β2MG level and age (years) or BL (m) were also determined by polynomial regression analysis, and the reference serum β2MG level was expressed as a cubic equation of age or BL (Fig. 1a, b; broken lines). The regression equations were as follows:

Table 1 Median, 2.5th percentile, and 97.5th percentile of serum β2MG reference values in each age group according to sex

Age	All subjects				Boys				Girls			
	n	2.5 %	50 %	97.5 %	*n*	2.5 %	50 %	97.5 %	*n*	2.5 %	50 %	97.5 %
3–5 months	21	1.5	1.8[a]	3.2	17	1.5	1.8	3.2	4	1.6	1.8	2.1
6–8 months	18	1.4	1.8[a]	2.6	14	1.4	1.9	2.6	4	1.6	1.6	2.3
9–11 months	29	1.3	1.7[a]	3.3	15	1.3	1.7	3.3	14	1.3	1.8	3.2
1 years	69	1.4	1.7[a]	3.1	32	1.4	1.7	3.2	37	1.2	1.6	3.0
2 years	73	1.0	1.5	2.5	40	1.0	1.5	2.2	33	1.0	1.5	3.4
3 years	85	1.0	1.5	2.3	46	1.1	1.5	2.3	39	1.0	1.5	2.4
4 years	78	1.1	1.4	2.5	42	1.0	1.4	2.1	36	1.1	1.4	3.1
5 years	94	1.1	1.4	2.3	46	1.1	1.5	2.7	48	1.0	1.4	2.2
6 years	101	1.1	1.4	2.3	43	1.1	1.4	2.4	58	1.0	1.5	2.3
7 years	83	1.0	1.4	2.1	36	0.9	1.3	2.1	47	1.0	1.4	2.2
8 years	55	1.0	1.4	2.5	19	1.0	1.4	1.8	36	1.0	1.4	2.3
9 years	37	1.0	1.4	2.1	18	1.1	1.4	1.8	19	1.0	1.4	2.1
10 years	42	0.9	1.3	1.9	11	1.1	1.4	1.6	31	0.9	1.3	1.9
11 years	58	1.0	1.3	2.3	19	1.1	1.3	2.1	39	1.0	1.2	2.4
12 years	69	1.0	1.3	1.8	14	1.2	1.3	1.5	55	0.9	1.3	1.9
13 years	68	1.0	1.3	1.8	30	1.0	1.4	2.0	38	1.0	1.2	1.5
14 years	57	0.9	1.3	2.0	17	1.1	1.4	2.0	40	0.9	1.2	1.7
15 years	35	0.8	1.2	1.8	15	0.8	1.2	1.8	20	0.8	1.1	1.7
16 years	59	0.8	1.2	1.8	30	0.8	1.2	1.8	29	0.8	1.1	1.4
All ages	1311	1.0	1.4	2.3	504	1.0	1.4	2.3	627	1.0	1.4	2.3

[a] $P < 0.0001$ in comparison to the mean value in all subjects

For age: $y = -0.000472x^3 + 0.0139x^2 - 0.149x + 1.94$

For BL: $y = -0.354x^3 + 1.79x^2 - 3.26x + 3.36$

β2MG exhibited significant correlations with age (correlation coefficient of -0.50) and with BL (correlation coefficient of -0.49), which were slightly improved compared to those in the linear regression analysis.

There was no relationship between the β2MG concentration and age in children less than 2 years of age; however, β2MG levels showed a significant negative correlation with age in children more than 2 years of age (Fig. 1c, d). Statistical analyses revealed that the β2MG levels in age groups of 0–5 months (1.94 ± 0.44 mg/l), 6–8 months (1.92 ± 0.38 mg/l), 9–11 months (1.80 ± 0.48 mg/l), and 1 year (1.80 ± 0.42 mg/l) were significantly higher than the overall mean value of all the subjects (1.45 ± 0.34 mg/l, $P < 0.001$). However, no difference was found in the >2 years age group.

There were 14 outliers of the upper limit of age-specific values (Fig. 2a); however, these were unrelated to the corresponding Cr values, which were within the normal range (Fig. 2b). Out of the 14 children, 6 were taking cold medicine or antiallergic agents, and the number of subjects taking such medicines was significantly high (66 cases) among the total subjects ($P < 0.001$, by the chi-square test).

Assessment of β2MG value in children with CKD

The validity of the reference range of the established reference value for β2MG was tested by reviewing data from children with various kidney diseases during the course of

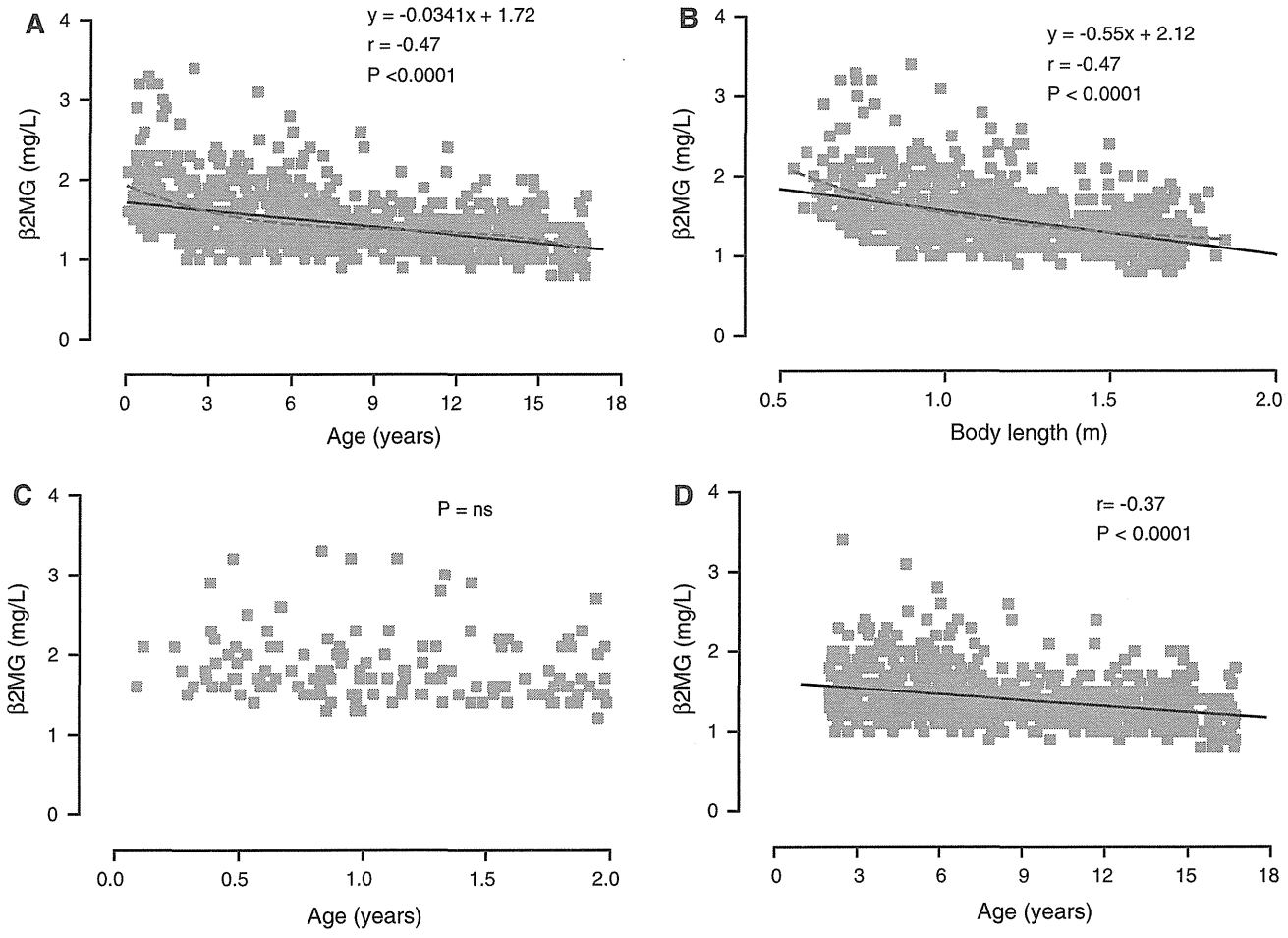

Fig. 1 Serum concentrations of β2MG in relation to age and body length (BL). Linear regression lines between the serum concentration β2MG and age (year) (**a**) or BL (m) (**b**) of all subjects are shown. The regression equations are $y = -0.0341x + 1.72$ and $y = -0.0055x + 2.12$, respectively (*straight lines*). The relationships are also determined by polynomial regression analysis, and the reference serum β2MG level is expressed as a cubic equation of age (**a**) or BL (**b**) (*broken lines*). The regression equations are as follows: $y = -0.000472x^3 + 0.0139x^2 - 0.149x + 1.94$ for age and $y = -0.354x^3 + 1.79x^2 - 3.26x + 3.36$ for BL. β2MG did not correlate with ages less than 2 years (**c**), but it did correlate significantly with ages above 2 years (**d**)

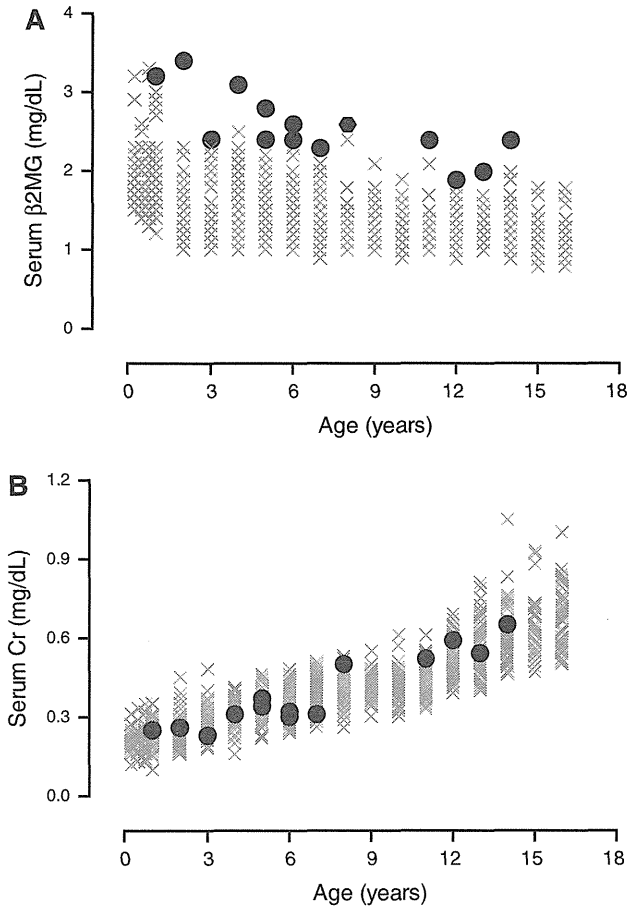

Fig. 2 Age-specific serum concentrations of β2MG (**a**) and Cr (**b**). Outliers beyond the 97.5th percentile range for β2MG reference values (**a**) and their corresponding Cr values (**b**) are shown as *black dots*

their disease. Most of the serum concentrations of β2MG were beyond the upper 97.5th percentile of age-appropriate reference values when the Cr level was beyond the 97.5th percentile of age-appropriate reference values. Out of the 345 samples, 329 indicated reduced kidney function below 75 %, and 344 of these (99.7 %) could be detected using the newly established age-specific β2MG reference range. However, data from 2 patients showed a discrepancy between the serum β2MG concentrations and serum Cr level or kidney function (Fig. 3). Their kidney function as evaluated based on the serum Cr value and BL was gradually decreased from the normal level to below 75 % during their course, but it was accompanied by a relatively quick increase of β2MG for their age (Fig. 3).

Patient 1 was a 14-year-old boy and was referred to the department of pediatric nephrology for proteinuria and severe emaciation. He had been diagnosed with mitochondrial disease by a muscle biopsy when he was 11 years old. His body weight was 21.1 kg (−3.0 SD for mean Japanese weight at his age) and body length was 136.5 cm (−3.6 SD). Laboratory data showed proteinuria, 120 mg/dl without kidney insufficiency; serum Cr, 0.42 mg/dl; and β2MG, 1.6 mg/l. His BL gradually increased to 143.8 cm (−4.4 SD) over the next 2 years, but his body weight was stable at 20 kg (−3.9 SD). The serum β2MG level gradually increased with the decrease of kidney function and exceeded the upper limit (97.5th percentile) of the established standard range for his age when he was 15.6 years old (Fig. 3a). At that time, an endogenous Cr clearance (CCr) test revealed his CCr to be 53.0 ml/min/1.73 m^2.

Patient 2 was a boy diagnosed with FSGS when he was 13 years old. At diagnosis, his serum Cr and β2MG levels were 0.5 mg/dl and 1.9 mg/l, respectively. His BL was 144.6 cm (−1.6 SD for the mean Japanese BL at his age) and calculated kidney function was 110.3 %. In addition to FSGS, he had an uncontrolled nephrotic range of proteinuria, and his kidney function decreased below 75 % in the next 9 months (Fig. 3b). His serum levels of both Cr and β2MG were elevated according to his kidney function, and the β2MG level was beyond the upper 97.5th percentile range during the same time that the kidney function decreased below 75 %. In contrast, his serum Cr level was still within the normal range for his age when the calculated kidney function decreased below 75 % (Fig. 3b).

Discussion

Several serum markers, including Cr, β2MG, and cystatin C, have been used to evaluate kidney function [7, 8]. However, for the use of these markers in children, an understanding of their normal reference values and their relationships with age and build according to differences among races is essential. Therefore, we recently conducted an ongoing multicenter large-scale study to examine this point. The reference value for serum Cr in healthy Japanese children has already been established [1]. The present study was aimed at determining the reference range of β2MG in healthy Japanese children as the second step of our study.

In this study, we found a significant correlation between β2MG concentration and age (Fig. 1a), which was different from previous reports [4, 5]. There was also a significant negative correlation between β2MG and BL, and they had the same regression coefficient ($r = -0.47$) (Fig. 1b). Therefore, it can be argued that the independent relation of β2MG with age and body mass, which has been one of the advantages for its use as a marker, is not applicable in studies on children. However, the current study showed that the slope of the regression line for β2MG with age is gradual and reaches a plateau in a short time (Fig. 1). Moreover, β2MG and age are negatively correlated, and therefore, elevations in β2MG concentrations relative to age can be easily detected. Indeed, the retrospective

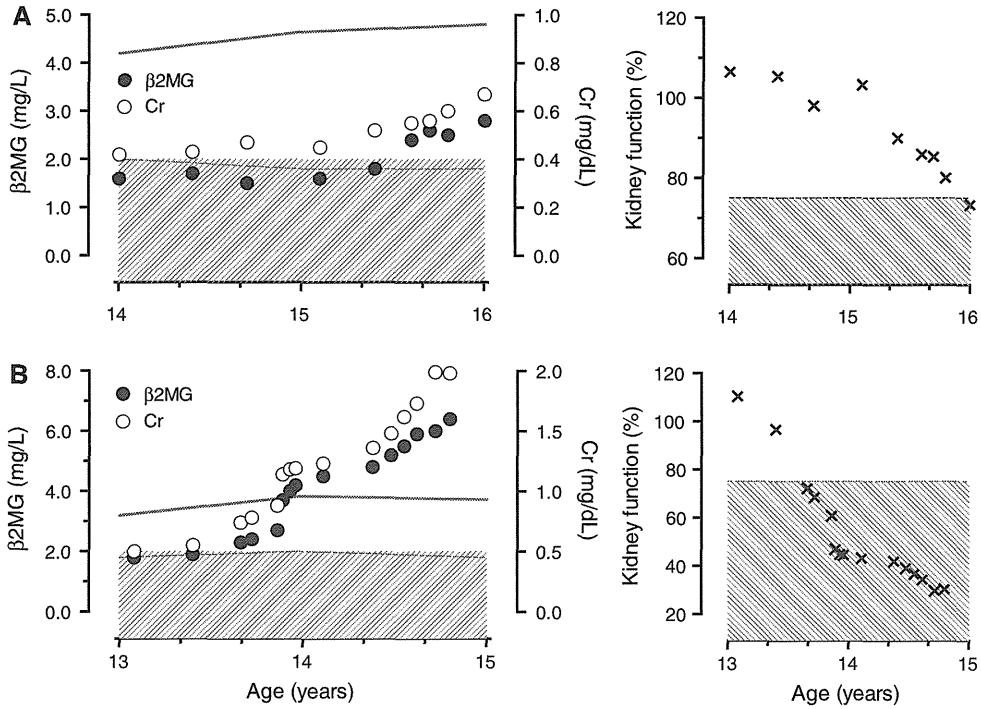

Fig. 3 Time course of kidney function (%) and serum β2MG and Cr concentrations in patient 1 (**a**) and patient 2 (**b**). *Shaded area* and *solid line in the left panel* represent the age-specific reference range (2.5–97.5th percentile) for β2MG and Cr, respectively. The *shaded area in the right panel* represents the age-specific reference range for kidney function under 75 %

assessments tested the clinical validity of the newly established β2MG reference in patients with various kidney diseases, distributed over a wide range of age groups, revealed that β2MG was a highly sensitive marker (99.7 %) for detecting kidney dysfunction below 75 %.

β2MG forms the beta chain of the human leukocyte antigen class I molecule and is present on the surface of most nucleated cells [9]. Although the mechanism of the dependency of β2MG on age is unknown, many immunological features in children, including an immature immune system in infants and lymphocytic predominance of circulating leukocytes in young children, could explain how serum β2MG concentrations change with age. Many of the subjects among the high β2MG outliers were taking cold medicine or antiallergic agents, indicating that some kind of immune reaction caused by the common cold or some allergic diseases, including bronchial asthma and atopic dermatitis, could affect β2MG production. Indeed, such diseases are common among young children. Data from studies examining serum β2MG values in fetuses or neonates reveal that the mean value of β2MG is relatively higher (around 3.5 mg/l) than that of young children with no renal complications in the present study [10, 11].

The current study used the equation for kidney function derived from serum Cr: kidney function (%) = (reference serum Cr/patient's serum Cr) × 100, since the reciprocal of serum Cr is generally correlated with GFR [12, 13]. Assuming 100 % kidney function to be GFR 120 ml/min, 75 % kidney function is comparable to GFR 90 ml/min, which is the borderline between CKD stage 1 and 2 [14]. An advantage of using this method is that since this formula is based on BL rather than age, kidney function can be appropriately estimated for growing children. There are, however, still significant disadvantages of using Cr as a marker for detecting mild impairment of kidney function in children. Herein, we presented a typical case of this situation (Fig. 3). In children with a very low muscle mass, Cr-based estimation of GFR can be misleading. Cr can also be overestimated in children with advanced renal failure, in whom there is reduced Cr production due to malnutrition [13]. Although β2MG has the disadvantage of being increased in patients with inflammatory and infectious diseases and several malignancies [6], detection of increased β2MG concentrations appears to be easier than that of Cr. Therefore, as compared to Cr, β2MG appeared to be a better marker of kidney impairment in children with abnormally low body mass. It also appears to be favorable for children with short stature in mild kidney dysfunction.

In addition to β2MG, recent studies have reported that cystatin C also facilitates the recognition of abnormal renal function in children compared to Cr because its reference range is independent of age, gender, height, and body composition [7, 8]. The applicability of cystatin C, however, remains a matter of debate. A standard value for cystatin C in children has not yet been established; therefore, considering the diagnostic sensitivity of cystatin C for impaired GFR in pediatric patients, particularly in patients with only mildly impaired kidney function, cystatin C may not be a better indicator than the BL/Cr ratio [15]. Furthermore, the measurement of cystatin C is currently too

expensive for routine use in clinical practice. However, cystatin C will also be a potentially useful marker once a reference value in normal children, according to race, has been established, and the differences between the diagnostic significance of Cr and β2MG become clear. We believe that our ongoing large-scale study that aims to establish the reference value of cystatin C in Japanese children will provide a better understanding of this marker for clinical use.

In summary, the current study determined a new β2MG reference value for detecting kidney impairment in children. Measurement of the serum β2MG concentrations in combination with serum Cr concentrations, and perhaps cystatin C in the near future, as markers for predicting glomerular function will provide better accuracy in the detection of reduced kidney function in children.

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ORIGINAL ARTICLE

Reference ranges for serum cystatin C measurements in Japanese children by using 4 automated assays

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Abstract
Objective The data available on reference ranges for cystatin C in children are limited, and there are discrepancies among the available data. The aim of this study was to describe the reference ranges for cystatin C in Japanese children by using 4 automated assays.
Methods Serum cystatin C levels were measured in 1128 Japanese children aged 3 month to 16 years without kidney disease. We calculated age-, gender-, race- and assay-specific cystatin C ranges.
Results For all 4 assays, the median serum cystatin C levels were raised in term infants compared with older children and decreased by the first 2 years. The median serum cystatin C levels remained constant throughout up to the age of 14 years and decreased in children aged 15–16 years. The median serum cystatin C levels in children aged 12–16 years were slightly higher in males than in females. Assay-specific differences were also observed in the levels of serum cystatin C measured.
Conclusion Age-, gender-, race- and assay-specific ranges for serum cystatin C should be used as another tool to assess kidney function in children.

Keywords Cystatin C · Reference ranges · Children · Standardization

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Introduction

Serum creatinine is the most widely used marker to predict glomerular filtration rate. However, serum creatinine concentrations are not determined only by glomerular filtration [1], as creatinine production is proportional to muscle mass [2]. In children, muscle mass increases significantly with linear growth. To reflect the renal function, serum creatinine concentrations should be adjusted for body height and body size. In childhood, therefore, serum creatinine levels are dependent on age and muscle mass [3–6].

Cystatin C, a 13 kDa non-glycosylated low molecular weight protein [7], is a proteinase inhibitor involved in the intracellular catabolism of proteins [8]. Unlike creatinine, cystatin C is produced in all investigated nucleated cells at a constant rate, freely filtered in the renal glomeruli, and almost completely reabsorbed and catalyzed in the renal proximal tubular cells [9, 10].

In the existing literature, the proposed ranges for serum cystatin C in pediatric populations are inconsistent, with several small, single-institution, hospital, or clinic-based studies [11, 12]. In addition, the reported cystatin C ranges are affected by use of different cystatin C assays [13]. Furthermore, some previous studies suggested that cystatin C levels were independent of gender, age and body composition [14, 15], whereas others showed differences in serum cystatin C levels according to gender, age and race

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[16]. The aim of this study was to establish reference ranges for cystatin C levels in Japanese children by using 4 different assays.

Subjects and methods

Serum cystatin C levels were studied in 1128 children (503 boys and 625 girls) aged between 3 months and 16 years visiting the outpatient pediatric clinic, or hospitalized at Aichi Children's Health and Medical Center, Tokyo Metropolitan Children's Medical Center, Yokohama City University Medical Center, Niigata University, Seirei Hamamatsu General Hospital, Fussa Hospital, or Tokyo Health Service Association between 2008 and 2009 without clinical evidence of kidney diseases, urogenital diseases, infectious diseases, inflammatory diseases, muscular diseases, malignant diseases, cardiovascular diseases, liver or pancreas diseases, anomaly syndrome, hypertension, dehydration, or pregnancy. None of the subjects had hyperthyroidism or hypothyroidism. The children's parents provided written informed consent according to the Declaration of Helsinki, and ethics approval was obtained from the institutional review board.

Serum cystatin C was analyzed at SRL Inc (Tokyo, Japan) by using 4 different cystatin C assays—Nescaute GC cystatin C (Alfresa Pharma Corporation, Osaka, Japan), LZ TEST 'EIKEN' cystatin C (Eiken Chemical, Tokyo, Japan), and Iatro Cys-C (Mitsubishi Chemical Medience, Tokyo, Japan) on the BioMajesty JCA-BM8020, and the N Latex Cystatin C assay (Siemens Healthcare Diagnostics Inc., Tokyo, Japan) on the Behring Nephelometer II (BNII; Siemens Healthcare Diagnostics Inc., Tokyo, Japan). All assays were programmed and calibrated according to the manufacturer's instructions.

The central 95 % reference ranges were calculated using the nonparametric method, and the Mann–Whitney U test was used for the analysis. All p values were based on two-sided testing and a significance level of 0.05 was used for the analysis.

Table 1 Patient characteristics

Characteristic	Age (years)	Median (interquartile range)
Height (cm)	0–1	74.0 (69.0–80.5)
	2–5	100.2 (93.0–106.3)
	6–11	124.2 (117.0–136.3)
	12–14	
	Male	160.2 (154.7–165.4)
	Female	155.0 (151.8–159.0)
	15–16	
	Male	169.3 (164.1–172.5)
	Female	159.2 (155.7–162.6)
Weight (kg)	0–1	9.0 (8.0–10.4)
	2–5	15.4 (13.5–17.7)
	6–11	25.0 (21.0–32.0)
	12–14	
	Male	49.5 (42.1–57.5)
	Female	46.9 (43.4–52.1)
	15–16	
	Male	56.6 (52.1–61.8)
	Female	50.7 (47.3–55.8)
Body mass index (kg/m^2)	0–1	16.4 (15.6–17.4)
	2–5	15.6 (14.8–16.4)
	6–11	16.0 (14.8–17.5)
	12–14	
	Male	19.0 (17.0–21.7)
	Female	19.8 (18.0–21.5)
	15–16	
	Male	19.7 (18.5–21.8)
	Female	20.2 (19.0–21.7)
Serum creatinine (mg/dL)	0–1	0.22 (0.19–0.25)
	2–5	0.29 (0.25–0.33)
	6–11	0.39 (0.34–0.44)
	12–14	
	Male	0.59 (0.54–0.66)
	Female	0.55 (0.49–0.60)
	15–16	
	Male	0.71 (0.66–0.81)
	Female	0.58 (0.54–0.64)

Results

Subject characteristics are shown in Table 1. The subjects' median height and weight were 117.6 cm (range 57.0–184.6), and 21.7 kg (range 5.0–100.8 kg), respectively. The median body mass index (BMI) of the subjects was 16.4 (range 12.2–32.5) and 2 (0.2 %) of 1128 subjects had a BMI of $\geq$30.

The serum concentrations of cystatin C were highest after birth followed by a decrease over the following months in each assay, when normal adult ranges of cystatin C were reached (Fig. 1). After the first 2 years of life, the median serum cystatin C became constant and slightly decreased in children aged 15–16 years. The median serum cystatin C level in children aged 2–11 years was similar in males and females ($p \geq 0.05$; all assays). However, the median serum cystatin C level in children aged 12–16 years was significantly higher in males than in females ($p < 0.0001$; all assays) (Fig. 2).

The distribution of serum cystatin C for children by age, gender and assay is shown in Table 2. The reference ranges in children aged 2–11 years were Alfresa, 0.59–1.01 mg/L;

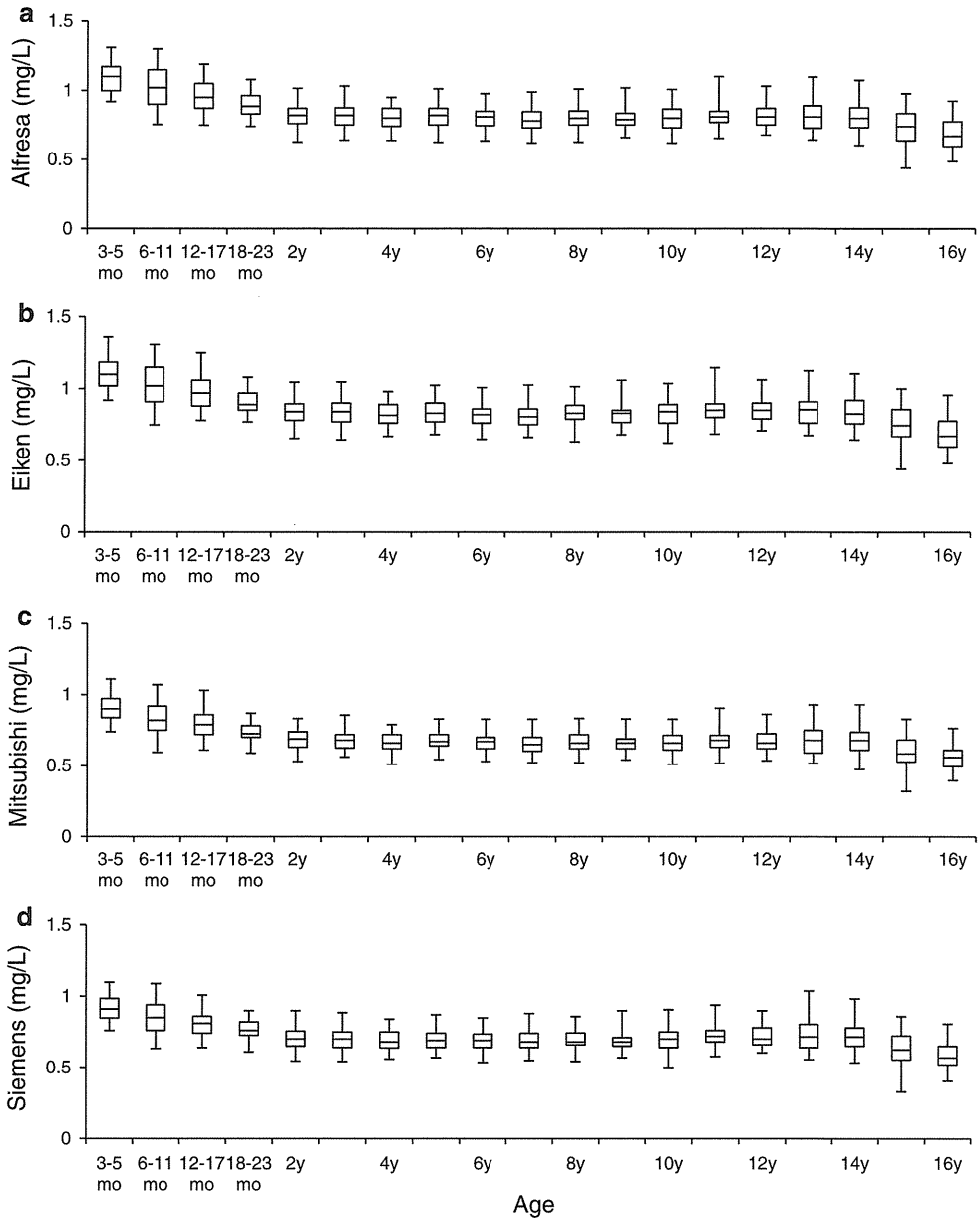

Fig. 1 Serum cystatin C in children aged 3 months to 16 years. The box plot extends from the 25th percentile to the 75th percentile, with the horizontal line at the median, and the whiskers show the central 95 % of the data for Alfresa (**a**), Eiken (**b**), Mitsubishi (**c**), and Siemens assays (**d**)

Eiken, 0.61–1.04 mg/L; Mitsubishi, 0.50–0.83 mg/L; and Siemens, 0.52–0.88 mg/L. Overall, the serum cystatin C levels measured using the Alfresa and Eiken assays were significantly higher than those measured using the Mitsubishi and Siemens assays ($p < 0.0001$).

Discussion

Serum cystatin C concentrations were measured in children by using 4 different automated assays and calculated assay-specific cystatin C ranges in this study. The highest serum cystatin C concentration measured by all 4 assays was found after birth, followed by a rapid decrease over the following months, consistent with previously published data [17, 18]. Cataldi et al. [19] reported that serum cystatin C does not cross the placental barrier; therefore, the high values of serum cystatin C after birth probably reflect the degree of maturation of the glomerular filtration capacity.

The concentrations of serum cystatin C were constant in children >2 years, and the nonparametric reference ranges of the Alfresa and Eiken assays were higher than that obtained by the Mitsubishi and Siemens assays. The difference had been explained by the differences in the methods for measurement of a particle-enhanced nephelometric immunoassay in contrast to a particle-enhanced turbidimetric immunoassay [20]. However, since the Eiken assay and the Mitsubishi assay are both particle-enhanced turbidimetric immunoassays, the difference of

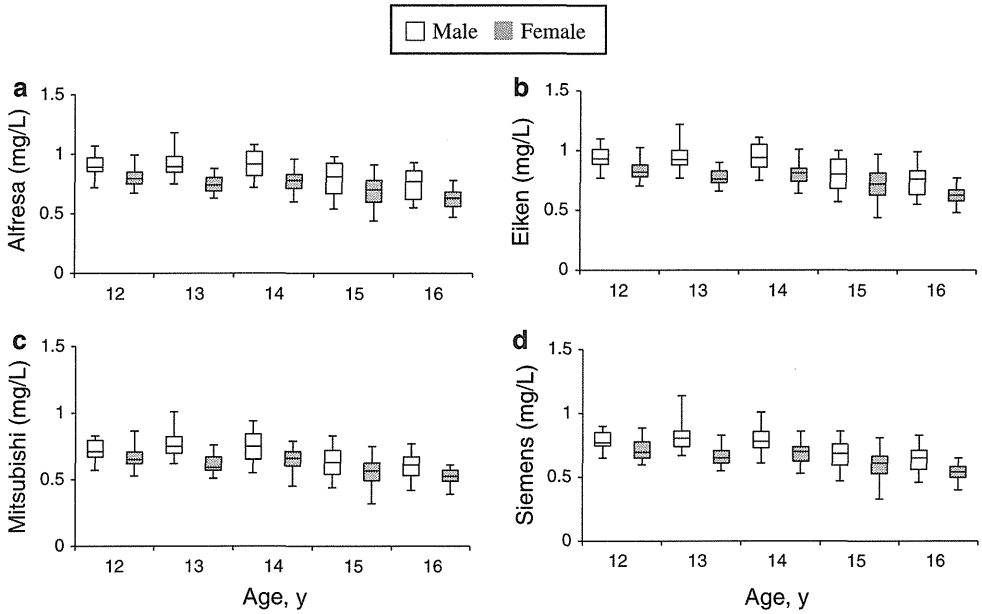

Fig. 2 Serum cystatin C in male and female children. The box plot extends from the 25th percentile to the 75th percentile, with the horizontal line at the median, and the whiskers show the central 95 % of the data for Alfresa (**a**), Eiken (**b**), Mitsubishi (**c**), and Siemens assays (**d**)

Table 2 The central 95 % reference ranges and median of serum cystatin C (mg/L) measured by the 4 assays in children

Age	*n*	Alfresa			Eiken			Mitsubishi			Siemens		
		2.5 %	50.0 %	97.5 %	2.5 %	50.0 %	97.5 %	2.5 %	50.0 %	97.5 %	2.5 %	50.0 %	97.5 %
3–5 months	18	0.92	1.10	1.31	0.92	1.10	1.36	0.74	0.90	1.11	0.76	0.91	1.10
6–11 months	47	0.75	1.02	1.30	0.75	1.02	1.31	0.59	0.82	1.07	0.63	0.85	1.09
12–17 months	31	0.75	0.95	1.19	0.78	0.97	1.25	0.61	0.79	1.03	0.64	0.81	1.01
18–23 months	38	0.74	0.89	1.08	0.77	0.89	1.08	0.59	0.73	0.87	0.61	0.76	0.90
2–11 years	704	0.64	0.81	0.99	0.67	0.83	1.02	0.53	0.67	0.83	0.56	0.69	0.86
12–14 years	191	0.65	0.81	1.07	0.67	0.85	1.08	0.52	0.67	0.89	0.57	0.71	0.92
Male	59	0.72	0.90	1.13	0.76	0.93	1.17	0.56	0.74	0.97	0.63	0.78	1.08
Female	132	0.63	0.78	0.96	0.66	0.81	1.00	0.51	0.64	0.80	0.55	0.69	0.86
15–16 years	99	0.49	0.70	0.96	0.48	0.70	0.99	0.40	0.57	0.79	0.41	0.60	0.85
Male	47	0.54	0.78	0.98	0.55	0.78	1.00	0.42	0.61	0.82	0.46	0.65	0.86
Female	52	0.45	0.65	0.91	0.45	0.67	0.95	0.34	0.55	0.75	0.35	0.56	0.80
Adult													
Male			0.63–0.95			0.59–1.03			0.5–0.9			0.53–0.95	
Female			0.56–0.87										

the reference ranges for cystatin C was not explained by the use of the different methodologies.

This study showed that cystatin C decreased in children aged 15–16 years, and serum cystatin C in children aged 12–16 years was higher in males than in females, and supported the result of a previous study conducted in US adolescents [21]. In addition, assay-specific differences in serum cystatin C levels in children were also observed in this study. There are concerns raised with regard to measuring serum cystatin C levels, as assay-specific differences were observed in levels of serum cystatin C measured.

The Institute for Reference Materials and Measurements (IRMM) announced the availability of the new certificated reference material ERM-DA471/IFCC [22]. The standardized measurement of serum cystatin C using ERM-DA471/IFCC is now being developed.

In conclusion, our study provided age-, gender- and assay-specific ranges of cystatin C for Japanese children.

Age-, gender-, race- and assay-specific ranges for serum cystatin C should be used as another tool to assess kidney function in children. The standardized measurement of serum cystatin C will be a reliable marker for the recognition of abnormal renal function compared to serum creatinine.

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