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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

[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;

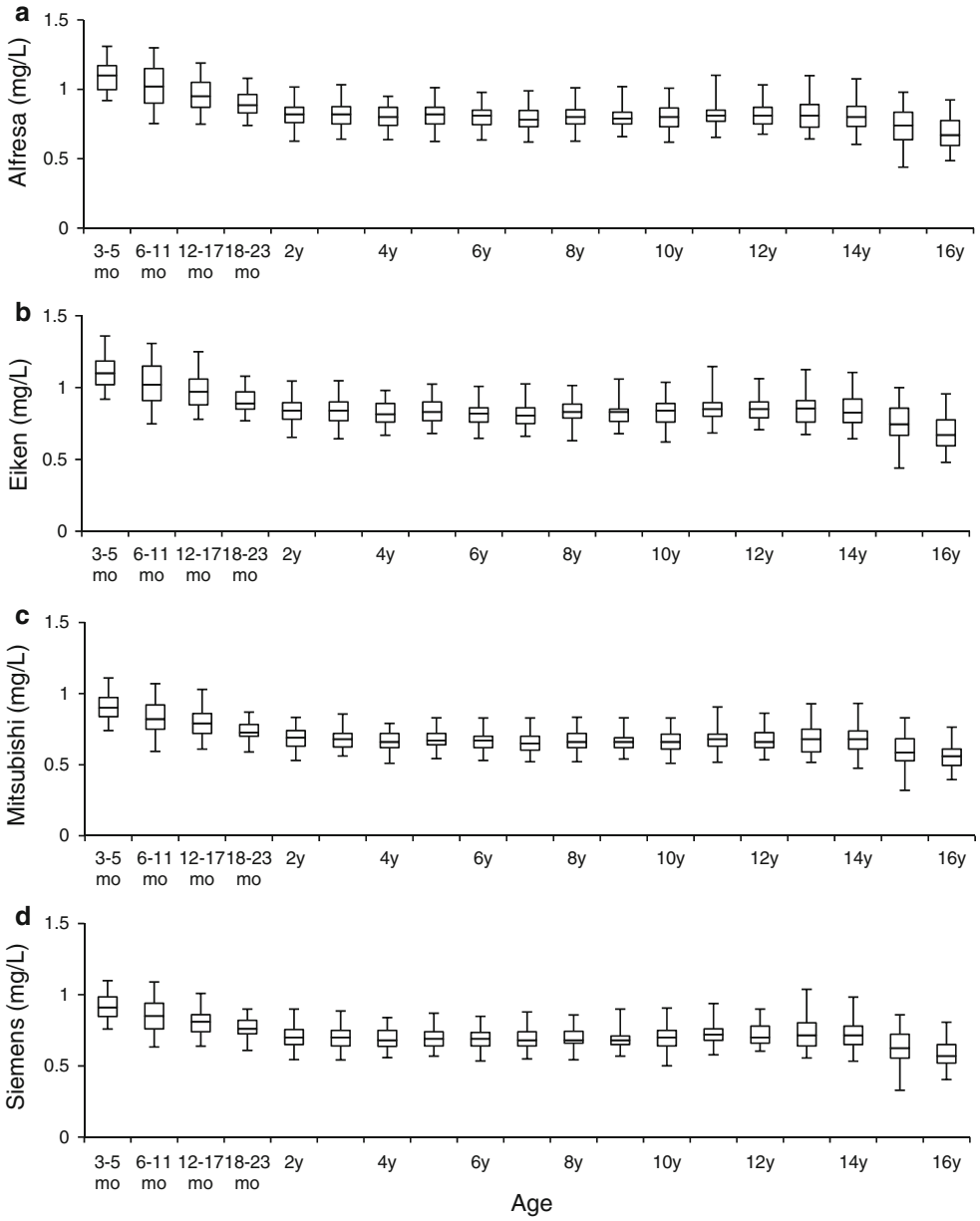

Fig. 1 Serum cystatin C in children aged 3 months to 16 years. The box plot extends from the 25th percentile to the 75th percentile, with the horizontal line at the median, and the whiskers show the central 95 % of the data for Alfresa (**a**), Eiken (**b**), Mitsubishi (**c**), and Siemens assays (**d**)

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

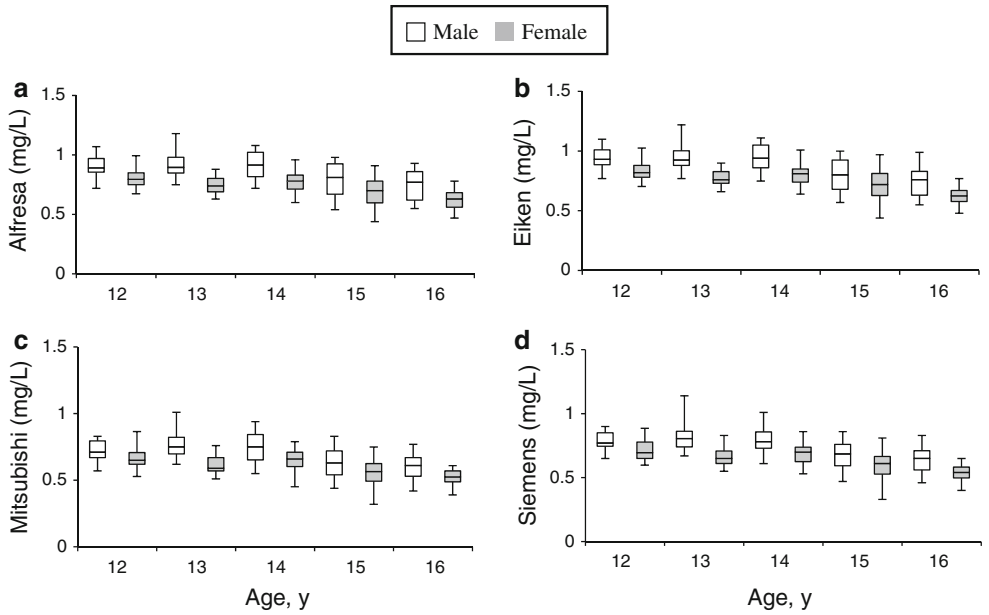

Fig. 2 Serum cystatin C in male and female children. The box plot extends from the 25th percentile to the 75th percentile, with the horizontal line at the median, and the whiskers show the central 95 % of the data for Alfresa (**a**), Eiken (**b**), Mitsubishi (**c**), and Siemens assays (**d**)

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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References

1. Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. Kidney Int. 1985;28:830–8.
2. Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem. 1992;38:1933–53.
3. Morris MC, Allanby CW, Toseland P, Haycock GB, Chantler C. Evaluation of a height/plasma creatinine formula in the measurement of glomerular filtration rate. Arch Dis Child. 1982;57:611–5.
4. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. Pediatr Clin North Am. 1987;34:571–90.
5. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, Nagai T, Ikezumi Y, Fujita N, Ito S, Iijima K, Kitagawa T. Age, gender, and body length effects on reference serum creatinine level determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol. 2011;15:694–9.
6. Uemura O, Ushijima K, Nagai T, Yamada T, Hayakawa H, Shinkai Y, Kuwabara M. Reference serum creatinine levels determined by an enzymatic method in Japanese children: relationship to body length. Clin Exp Nephrol. 2009;13:585–8.
7. Grubb A, Lofberg H. Human γ-trace. Structure, function and clinical use of concentration measurements. Scand J Clin Lab Investig. 1985;45:7–13.
8. Olafsson I. The human cystatin C gene promoter: functional analysis and identification of heterogeneous mRNA. Scand J Clin Lab Investig. 1995;55:597–607.
9. Grubb A. Diagnostic value of analysis of cystatin C and protein HC in biological fluids. Clin Nephrol. 1999;238:20–7.
10. Tenstad O, Roald AB, Grubb A, Aukland K. Renal handling of radiolabelled human cystatin C in the rat. Scand J Clin Lab Invest. 1996;56:409–14.
11. Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Brodehl J. Reference values for cystatin C serum concentrations in children. Pediatr Nephrol. 1998;12:125–9.
12. Finney H, Newman D, Thakkar H, Fell JM, Price CP. Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. Arch Dis Child. 2000;82:71–5.
13. Li J, Dunn W, Breaud A, Elliott D, Sokoll LJ, Clarke W. Analytical performance of 4 automated assays for measurement of cystatin C. Clin Chem. 2010;56:1336–9.
14. Bökenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. Cystatin C—a new marker of glomerular filtration rate in children independent of age and height. Pediatrics. 1998;101:875–81.
15. Helin I, Axenram M, Grubb A. Serum cystatin C as a determinant of glomerular filtration rate in children. Clin Nephrol. 1998;49:221–5.
16. Stevens LA, Schmid CH, Greene T, Li L, Beck GJ, Joffe MM, Froissart M, Kusek JW, Zhang YL, Coresh J, Levey AS. Factors other than glomerular filtration rate affect serum cystatin C levels. Kidney Int. 2009;75:652–60.
17. Filler G, Witt I, Priem F, Priem F, Errich JH, Jung K. Are cystatin C and β2-microglobulin better markers than serum creatinine for prediction of a normal glomerular filtration rate in pediatric subjects? Clin Chem. 1997;43:1077–8.
18. Uemura O, Ushijima K, Nagai T, Yamada T, Hayakawa H, Nabeta Y, Shinkai Y, Koike K, Kuwabara M. Reference serum cystatin C level in Japanese children. Clin Exp Nephrol. 2010;14:453–6.
19. Cataldi L, Mussap M, Bertelli L, Ruzzante N, Fanos V, Plebani M. Cystatin C in healthy women at term pregnancy and in their infant newborns: relationship between maternal and neonatal serum levels and reference values. Am J Prinatol. 1999;16:287–95.
20. Randers E, Krue S, Erlandsen E, Danielsen H, Hansen LG. Reference interval for serum cystatin C in children. Clin Chem. 1999;45:1856–8.
21. Groesbeck D, Köttgen A, Parekh R, Selvin E, Schwartz GJ, Coresh J, Furth S. Age, gender, and race effects on cystatin C levels in US adolescents. Clin J Am Nephrol. 2008;3:1777–85.
22. Grubb A, Blirup-Jensen S, Lindstrom V, Schmidt C, Althaus H, Zegers I. First certified reference material for cystatin C in human serum ERM-DA471/IFCC. Clin Chem Lab Med. 2010;48:1619–21.