研究計画書

濾紙血によるクレアチニン測定の信頼性の検討
「濾紙血を用いた新生児のクレアチニンスクリーニング検査研究」の予備研究

研究計画作成者

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Ⅰ．研究の背景

先天性腎尿路疾患(congenital abnormality of kidney and urinary tract:以下CAKUT)は全妊娠の0.5%に認められ、小児慢性腎不全の原因として最も多い。しかしCAKUTでは尿蛋白、尿潜血を呈するケースは少なく、CAKUTの発見につながりにくい。CAKUTの中には治療介入できない疾患も多いが、早期介入することで合併症予防や腎機能温存が可能な場合や、先行的腎移植の選択ができるようになるケースも存在する。CAKUTを早期に発見できることは有用であると考える。

CAKUTを効率良く発見するには画像診断法(特に超音波検査)の導入が最も望ましいが、これは施行者の技術の問題や対象が全出生児に及ぶことを考えると現実的ではない。

そこで、我々は新生児の血清クレアチニンを測定し、その基準値をもとにCAKUTの発見ができないか検討することとした。新生児期にはガスリー検査がすでに施行されているので、この濾紙法を利用した検査を行うこととした。現在新生児のマススクリーニングは従来ガスリー法が行われていたが、タンデムマス法の方がより多くの疾患をスクリーニングできるため、現在パイロットスタディが行われている。このタンデムマス法を用いればクレアチニンの測定も可能である。

新生児は出生後の血圧上昇と、プロスタグランジンなどの関与による腎血管抵抗の低下により、RPFがダイナミックに変動し、血清クレアチニンは大きく変化する。この点から新生児の血清クレアチニンの正常値に関しては、まだ基準値となるものがない。今回このクレアチニンの基準値を作成し、これから有意に逸脱するものに対象を絞って、精査を行うことでCKDの早期発見につなげたいと考える。これらにより、新生児期の採血で各個体の腎機能異常の児を発見することができれば、小児慢性腎臓病（ＣＫＤ）を早期に発見でき、早期治療により日本の小児ＣＫＤ対策に役立てることができると考えられる。

Ⅱ．研究の目的

従来の血清クレアチニン測定法と濾紙によるクレアチニン測定法のデータを比較し、十分な精度で相関があることを確認するのが目的である。

両者に相関があることが確認できれば、新生児マススクリーニングの際の濾紙血を用いて得られるクレアチニン値をもとに、新生児期の CAKUT を発見することにつながると考えられる。

Ⅲ．研究の方法

対象者の採血時に0.1mL追加採取し、タンデムマスと同様の手法でクレアチニンを抽出し、LC MS/MSで定量する。年齢、性別、身長、体重、血清クレアチニン値、ヘマトクリットを登録票に記載する。男女、年齢、疾患問わず計100検体を採取し、血清クレアチニン値と濾紙クレアチニン値の相関を評価する。

Ⅳ．研究の対象者

あいち小児保健医療総合センター腎臓科に入院した児。年齢、性別、基礎疾患は問わない。

Ⅴ．研究参加施設

あいち小児保健医療総合センター

Ⅵ．予定期間及び目標症例数

登録期間：2013 年 10 月 1 日～2014 年 12 月 31 日

目標症例数：100 例

Ⅶ．プライバシー保護に関する配慮

１．患者の同意：担当医師は研究開始に先立ち，患者に下記内容について文書を示し十分に説明した後，研究参加について本人または保護者から文書により同意を得る。尚，同意取得の年月日を同意書の所定の欄に記入する。

１） 研究の目的および方法

２） 患者が研究への参加に同意した場合でも随時これを撤回できること

３） 患者が研究への参加に同意しない場合であっても不利益は受けないこと

４） 研究が病院の倫理委員会または治験審査委員会(IRB)で審査・承認されていること

５） その他人権や個人情報の保護に関し必要な事項

２．患者の個人情報・機密保護

研究の実施においては患者氏名を研究症例番号により匿名化し，患者個人情報の機密保護について十分な配慮を行う。

Ⅷ．研究事務局

あいち小児保健医療総合センター

研究責任医師　あいち小児保健医療総合センター腎臓科　　上村　治

Ⅳ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書　籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
本田雅敬		編集主幹 本田雅敬	小児のCKD/AKI実践マニュアル －透析・移植まで－	診断と治療社	東京	2014	全冊
渡邊みお、仁科幸子	小児の診察、視反応、未熟児網膜症の診察	江口秀一郎	眼科外来処置・小手術クローズアップ	メジカルビュー	東京	2014	4-7

雑　誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yata N, Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Nagai T, Ikezumi Y, Fujita N, Ito S, Iijima K, Saito M, Keneko T, Kitagawa T.	Reference ranges for serum cystatin C measurements in Japanese children by using 4 automated assays.	Clin Exp Nephrol.	17	872-876	2013
Ikezumi Y, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, Nagai T, Fujita N, Ito S, Iijima K, Kaneko T, Uemura O	Establishment of a normal reference value for serum beta2 microglobulin in Japanese children : reevaluation of its clinical usefulness.	Clin Exp Nephrol.	17	99-105	2013
Uemura O, Nagai T, Ishikura K, Ito S, Hataya H, Gotoh Y, Fujita N, Akioka Y, Kaneko T, Honda M	Creatinine-based equation to estimate the glomerular filtration rate in Japanese children and adolescents with chronic kidney disease.	Clin Exp Nephrol		Epub ahead of print	2013
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Morimoto N, Ogiwara H, Miyazaki O, Kitamura M, Nishina S, Nakazawa A, Maekawa T, Morota N.	Gorham-Stout syndrome affecting the temporal bone with cerebrospinal fluid leakage.	Int J Pediatr Otorhinolaryngol	77(9)	1596-1600	2013
Azuma N, Ito M, Yokoi T, Nakayama Y, Nishina S.	Vitreous outcomes after early vitreous surgery for aggressive posterior retinopathy of prematurity.	JAMA Ophthalmol	131(10)	1309-1313	2013
本田雅敬	小児CKD患者の移行医療	日小児腎不全会誌	33	5-8	2013
本田雅敬	【学校検尿2013】学校検尿システム堅持に向けて　学校保健との関連から	小児科臨床	66(4)	797-804	2013
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服部元史，佐古まゆみ，金子徹治，永井明，芦田明，五十嵐隆，伊丹儀友，上田善彦，太田敏之，後藤芳充，里村憲一，平松美佐子，伊藤秀一，上村治，佐々木聡，波多江健，幡谷浩史，藤枝幹也，吉村仁志，秋岡裕子，石倉健司、濱崎裕子，大橋靖雄，本田雅敬	2006年～2011年末までの期間中に新規に発生した20歳未満の小児末期腎不全患者の実態調査報告	日児腎誌	26(2)	154-164	2013
仁科幸子，若山曉美，三木淳司，内海隆，羅錦營，林孝雄，臼井千恵，大月洋，宮田学，佐藤美保，三村治，木村亜紀子，菅澤淳，中村桂子，不二門尚	3D立体映像の視聴に関する実態調査：多施設共同研究.	日本眼科学会雑誌	117(12)	971-982	2013
仁科幸子	小児眼科手術と麻酔.	眼科手術	26(4)	521	2013
仁科幸子	小児眼科の最近の話題.	日本眼科学会雑誌	117(5)	415-417	2013
伊藤里美，仁科幸子	就学前のロービジョンケア.	あたらしい眼科	30(4)	431-435	2013
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Ⅴ. 研究成果の刊行物・別冊

Clin Exp Nephrol
DOI 10.1007/s10157-013-0784-x

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;

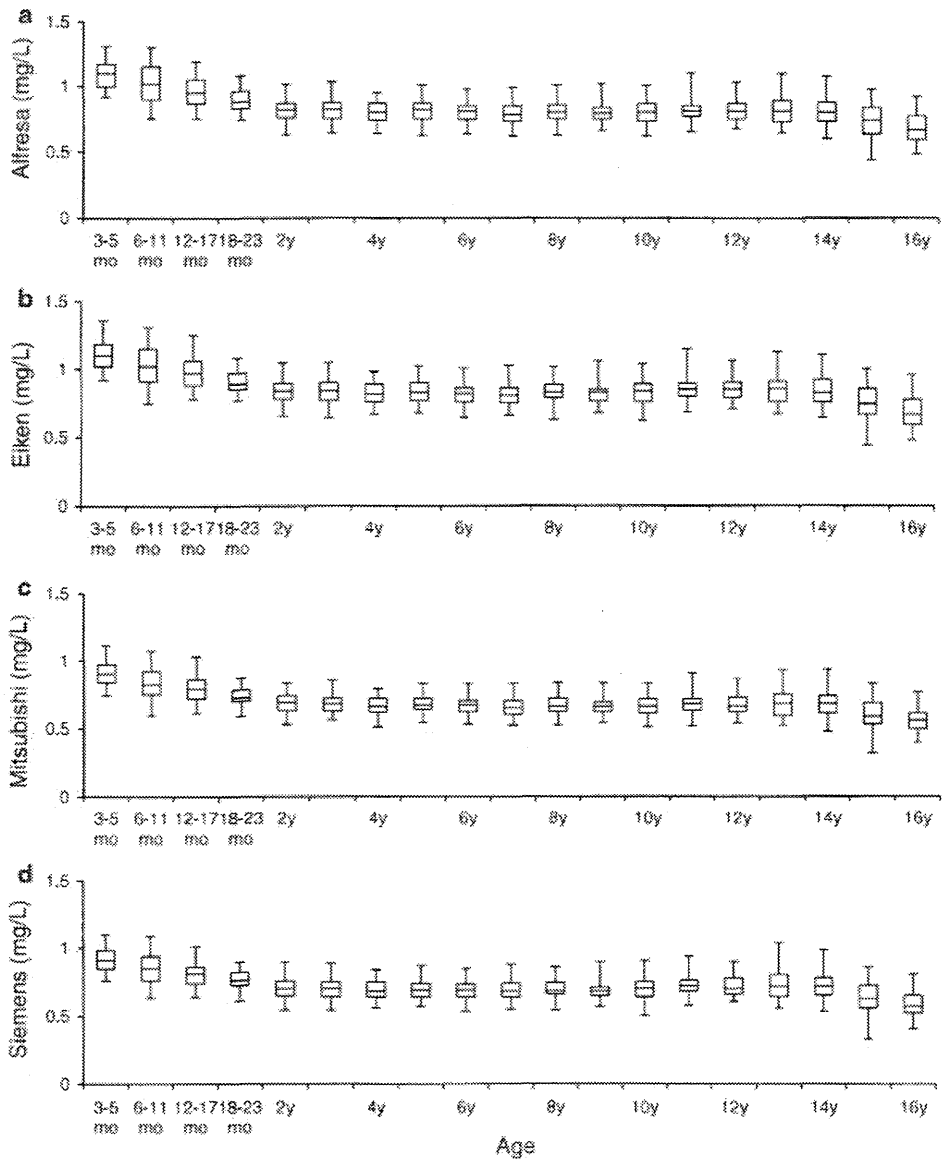

Fig. 1 Serum cystatin C in children aged 3 months to 16 years. The box plot extends from the 25th percentile to the 75th percentile, with the horizontal line at the median, and the whiskers show the central 95 % of the data for Alfresa (**a**), Eiken (**b**), Mitsubishi (**c**), and Siemens assays (**d**)

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

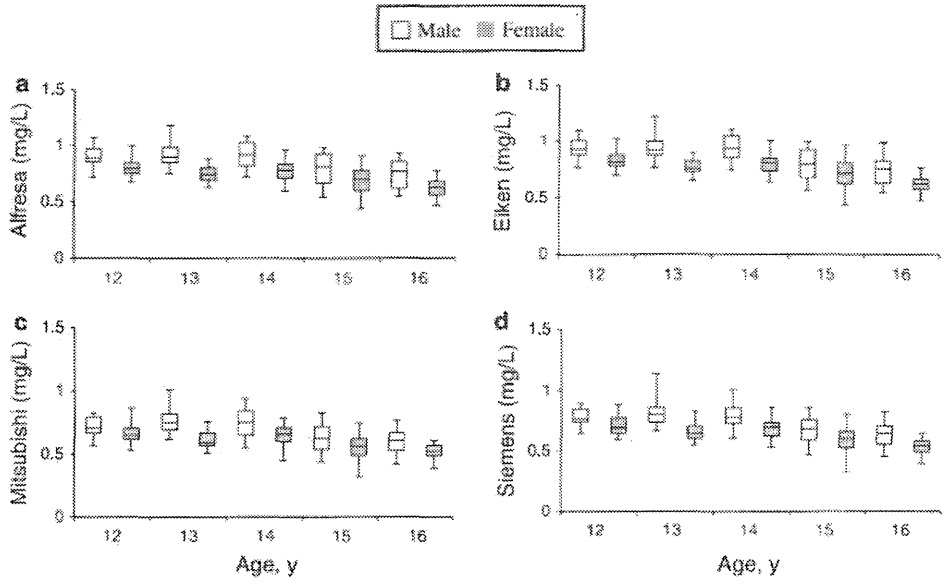

Fig. 2 Serum cystatin C in male and female children. The box plot extends from the 25th percentile to the 75th percentile, with the horizontal line at the median, and the whiskers show the central 95 % of the data for Alfresa (**a**), Eiken (**b**), Mitsubishi (**c**), and Siemens assays (**d**)

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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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

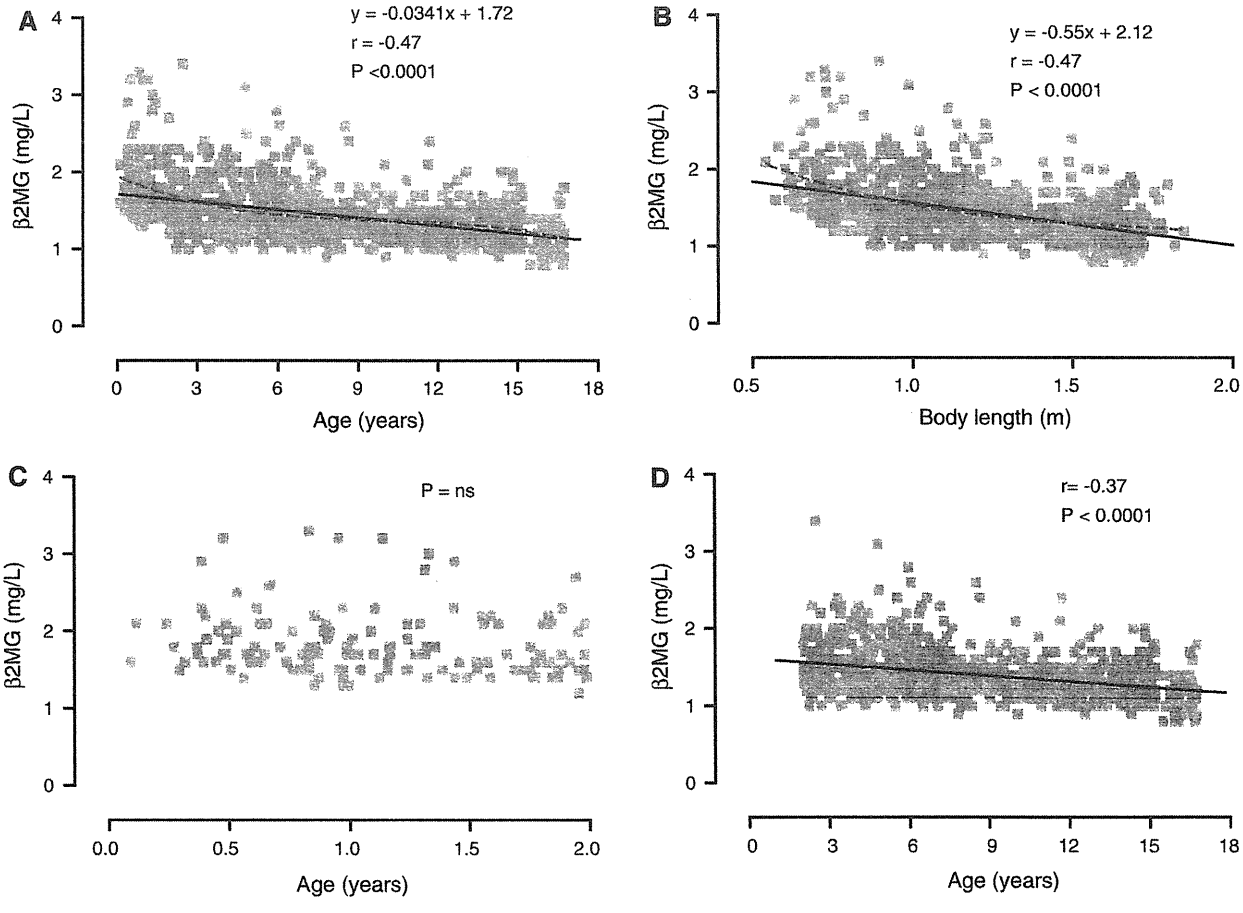

Fig. 1 Serum concentrations of β2MG in relation to age and body length (BL). Linear regression lines between the serum concentration β2MG and age (year) (**a**) or BL (m) (**b**) of all subjects are shown. The regression equations are $y = -0.0341x + 1.72$ and $y = -0.0055x + 2.12$, respectively (*straight 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**)

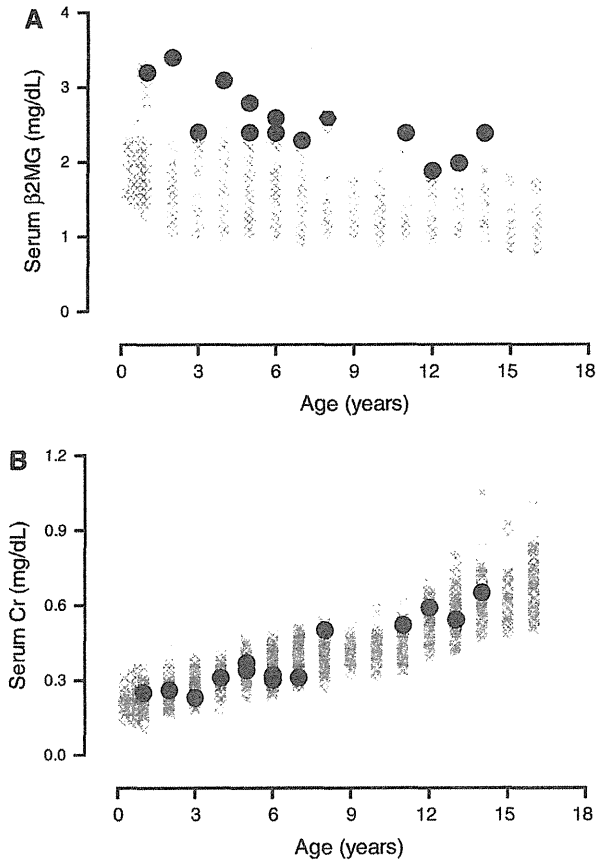

Fig. 2 Age-specific serum concentrations of β2MG (**a**) and Cr (**b**). Outliers beyond the 97.5th percentile range for β2MG reference values (**a**) and their corresponding Cr values (**b**) are shown as *black dots*

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

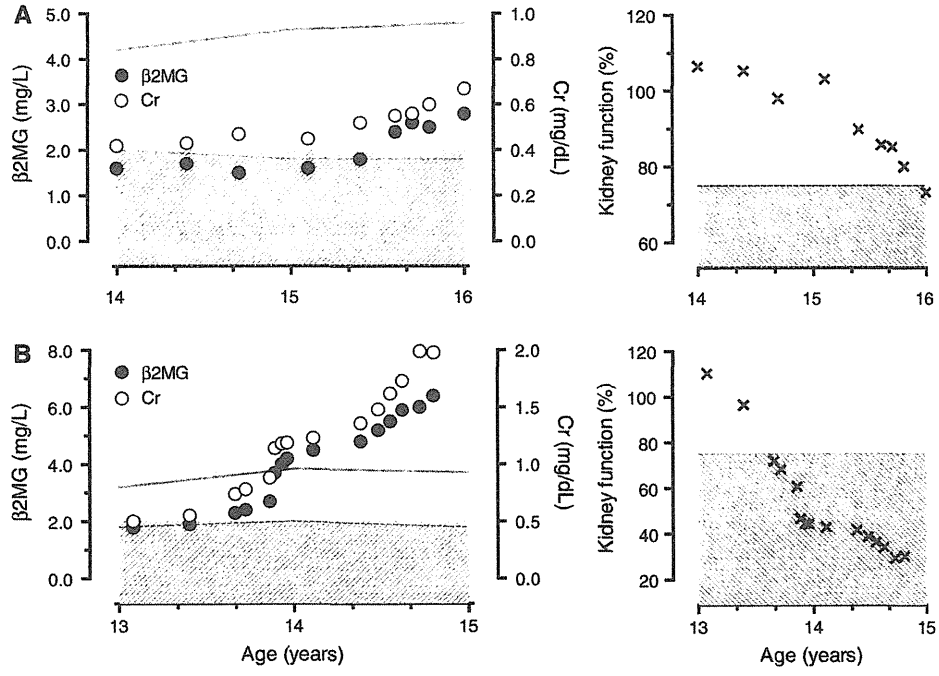

Fig. 3 Time course of kidney function (%) and serum β2MG and Cr concentrations in patient 1 (**a**) and patient 2 (**b**). *Shaded area* and *solid line in the left panel* represent the age-specific reference range (2.5–97.5th percentile) for β2MG and Cr, respectively. The *shaded area in the right panel*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