

Age- and sex-specific reference intervals for the serum cystatin C/creatinine ratio in healthy children (0–18 years old)

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Abstract

Objective: This study aimed to investigate serum levels of the cystatin C (CysC)/creatinine (Cr) ratio and renal serum markers (CysC, Cr, urea, and uric acid [UA]) for different ages and by sex. We also aimed to establish pediatric reference intervals for the serum CysC/Cr ratio.

Methods: Serum samples were collected from 4765 healthy children (0–18 years old). Serum markers of renal function were measured, and the CysC/Cr ratio of each participant was calculated and statistically analyzed.

Results: The renal marker CysC did not substantially change after 1 year old. Cr, urea, and UA levels generally increased with age. However, the serum CysC/Cr ratio steadily decreased with age. The CysC/Cr ratio showed significant differences in age among all age groups and varied with sex, except for in the 1 to 6-year-old groups. The overall serum CysC/Cr ratio in girls was higher than that in boys.

Conclusion: Reference intervals of the serum CysC/Cr ratio in the pediatric population were established. These intervals need to be partitioned by age and sex.

Keywords

Pediatric, serum marker, renal function, cystatin C/creatinine ratio, age, sex, reference interval

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Introduction

At present, serum creatinine (Cr) and cystatin C (CysC) levels are often used as reference indices for evaluating glomerular filtration function. However, serum Cr levels can be affected by non-renal factors, such as muscle mass, age, and sex.¹⁻³ CysC is less affected by non-renal factors compared with Cr.⁴ In patients with postrenal acute kidney injury, serum Cr levels are significantly increased, while there is only a slight increase in CysC levels.^{5,6} Determining whether the CysC/Cr ratio is a potential diagnostic marker for postrenal acute kidney injury would be helpful, especially for patients without definite hydronephrosis.⁷ The CysC/Cr ratio is considerably increased in a condition called “shrunken pore syndrome”, which is characterized by different glomerular filtration rate estimates based on serum Cr and CysC levels.⁸

Interestingly, some studies have reported that the serum CysC/Cr ratio might be an effective marker of kidney function in pediatric patients.⁹⁻¹¹ Serum CysC levels of pediatric patients show a wider range of changes than serum Cr levels,⁹ and they may be more sensitive to detect changes in renal function by the CysC/Cr ratio.¹⁰ Measurements of serum CysC levels lead to underestimation of renal dysfunction in pediatric patients.¹² The CysC/Cr ratio is preferable over use of any single marker in some chronic kidney diseases.⁹ In pediatric patients with acute renal failure, CysC levels only slightly increase without increasing Cr, suggesting that the CysC/Cr ratio reflects more delicate changes in the pediatric population.¹⁰

The above-mentioned studies show that the CysC/Cr ratio could be a potential biomarker for pediatric nephropathy. Therefore, establishing a biological reference interval of the CysC/Cr ratio, especially in healthy children, is necessary for diagnosing and treating pediatric kidney disease. Therefore, we

investigated pediatric serum CysC/Cr ratios and established reference intervals of the CysC/Cr ratio in healthy children (0–18 years old).

Methods

This study enrolled children (0–18 years old) who had a routine physical examination in the Department of Child Health at the Affiliated Hospital of Zunyi Medical University between June 2016 and March 2018. The exclusion criteria were as follows: 1) patients with nephropathy, digestive system diseases, acute and chronic infection, metabolic and nutritional diseases, rheumatic diseases, thyroid diseases, blood system diseases, heart diseases, malignant tumors, burns, muscle damage, obesity or weight loss, or hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg); 2) patients with maldevelopment or dysgnosia; and 3) patients with a history of surgery or blood transfusion. We divided subjects into seven groups according to age: <1 years (339 boys and 342 girls); 1 to 3 years (378 boys and 338 girls); 4 to 6 years (474 boys and 290 girls); 7 to 9 years (380 boys and 262 girls); 10 to 12 years (272 boys and 183 girls); 13 to 15 years (365 boys and 227 girls); and 16 to 18 years (503 boys and 412 girls). The participants' inclusion process is illustrated in Figure 1. Written informed consent for participation in the study or use of participants' blood was obtained from all participants (or their parent in the case of children aged younger than 16 years). All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the Affiliated Hospital of Zunyi Medical University (No. 2016(26)) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

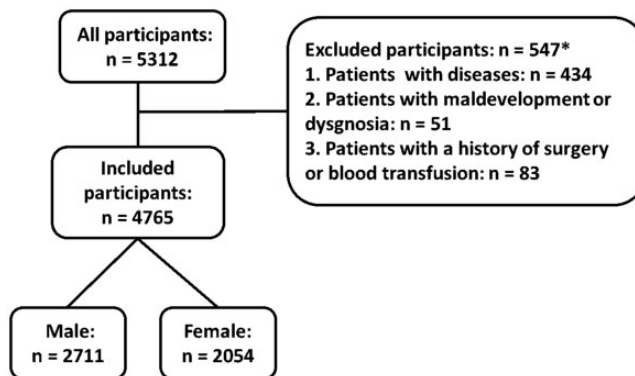


Figure 1. Flow chart showing all recruited participants, including those excluded from the study. *A total of 21 participants fulfilled two exclusion criteria: children with diseases or children with a history of surgery or blood transfusion.

The participants fasted for 6 to 12 hours before a health examination. After an informed consent form was signed, venous blood was collected in a tube (no additive), coagulated for 15 to 60 minutes, and centrifuged at $560 \times g$ for 10 minutes. The serum was obtained and stored at 0 to 8°C , and serum CysC, Cr, urea, and uric acid (UA) levels were determined within 6 hours. After analyses, the specimens were stored at -80°C for potential retesting.

The indirect immunofluorescence method was used to detect serum CysC levels (trueness: relative bias, 1.41%; precision: intra-assay precision, [Level 1] 1.96% and [Level 2] 1.87%, inter-assay precision, [Level 1] 2.56% and [Level 2] 2.48%). The serum CysC detection kit was purchased from Gensource Co., Ltd. (Shanghai, China) and the instrument parameters were set strictly in accordance with the reagent instructions. The CysC assay was calibrated using the Gensource self-contained calibrator (batch number: 0031A) and the matrix was human serum. Low-value and high-value serum quality control materials that were obtained from Gensource were used for quality controls. A sarcosine oxidase kit (Beckman Coulter, Suzhou, China) was used to detect serum Cr levels (trueness: relative bias, 1.09%;

precision: intra-assay precision, [Level 1] 0.51% and [Level 2] 0.54%, inter-assay precision, [Level 1] 1.77% and [Level 2] 2.22%). A urease–glutamate dehydrogenase kit (Beckman Coulter) was used to detect serum urea levels (trueness: relative bias, 0.22%; precision: intra-assay precision, [Level 1] 1.88% and [Level 2] 1.16%, inter-assay precision, [Level 1] 2.39% and [Level 2] 2.22%). A uricase–peroxidase kit (Beckman Coulter) was used to detect serum UA levels (trueness: relative bias, 0.28%; precision: intra-assay precision, [Level 1] 0.720% and [Level 2] 0.570%, inter-assay precision, [Level 1] 3.110% and [Level 2] 3.360%). The instrument parameters were set according to the instructions of the kits and the designated calibrator included in the Beckman Coulter test system (batch number: 66300) was used, with human serum as the matrix. Low-value and high-value human serum matrix standards from Bio-Rad (CA, USA) were used for quality control in the Cr, urea, and UA tests. The above-mentioned biochemical indices were measured on a Beckman Coulter AU5821 (Tokyo, Japan) biochemical analyzer and all of them were approved by the National Center for Clinical Laboratories external quality assessment programs in laboratory medicine.

Table 1. Median and interquartile range for each age group and sex.

Age, years	Median age of boys, interquartile range (years)	Median age of girls, interquartile range (years)	P
< 1	0.6 (0.4)	0.6 (0.4)	NS
1–3	2.3 (1.3)	2.3 (1.2)	NS
4–6	5.1 (1.3)	5.2 (1.3)	NS
7–9	8.1 (1.3)	8.1 (1.4)	NS
10–12	11.0 (1.4)	11.1 (1.3)	NS
13–15	14.2 (1.3)	14.1 (1.3)	NS
16–18	17.0 (1.3)	16.9 (1.4)	NS

P values were derived from comparison between sexes. NS: not significant.

Statistical analysis was performed using IBM SPSS Statistics 22.0 software (IBM Corp., Armonk, NY, USA). Logarithmic transformation was applied, and transformation to a normal population was unsuccessful. The Kruskal–Wallis test was used to compare the data between two groups. Differences with a value of $P < 0.05$ were considered to be statistically significant. The 95% reference intervals were then constructed using nonparametric analysis by following Clinical and Laboratory Standards Institute recommendations.¹³

Results

Among the participants, 2711 (56.9%) were boys, with a mean age of 9.11 ± 6.0 years, and 2054 (43.1%) were girls, with a mean age of 9.2 ± 6.3 years ($P > 0.05$). The participants were divided into seven age groups. The median age and interquartile ranges of each group are shown in Table 1.

The serum CysC/Cr ratio decreased with age, and significant differences were found among all age groups (all $P < 0.001$) (Figure 2a). The serum CysC/Cr ratios of girls aged < 1 year and those aged 7 to 18 years were significantly higher than those of boys (all $P < 0.01$) (Table 2). Serum CysC levels sharply decreased after 1 year old in boys and girls (both $P < 0.001$) (Figure 2b), and these levels remained stable thereafter. However, serum CysC levels in boys were

significantly higher than those in girls at 4 to 18 years old (all $P < 0.001$) (Table 2). Serum Cr levels were lowest in children aged 1 to 3 years and gradually increased with age after 3 years (Figure 2c), with significant differences among the different age groups in boys and girls (all $P < 0.001$) (Figure 2c). After the onset of adolescence (10–12 years old), Cr levels in boys increased more rapidly and became significantly higher than those in girls (both $P < 0.001$) (Table 2). In contrast to the trend in CysC levels, urea levels rapidly increased after 1 year old (all $P < 0.001$) (Figure 2d). However, consistent with the trend in CysC levels, after 1 year old, urea levels in boys were significantly higher than those in girls (all $P < 0.01$) (Table 2), but the differences between age groups were not significant (Figure 2d). Before the age of 12 years, there were no significant changes in UA levels (Figure 2e). However, after 12 years old, UA levels in boys rapidly increased and remained at approximately 6 mg/dL (Figure 2e), which was significantly higher than that of girls (both $P < 0.001$) (Table 2).

As mentioned above, significant differences were found in the serum CysC/Cr ratio among all age groups (all $P < 0.001$) (Figure 2a), but no sex difference was observed in the CysC/Cr ratio between healthy children aged 1 to 3 years old and those aged 4 to 6 years old (Table 2). Therefore, a statistical test was

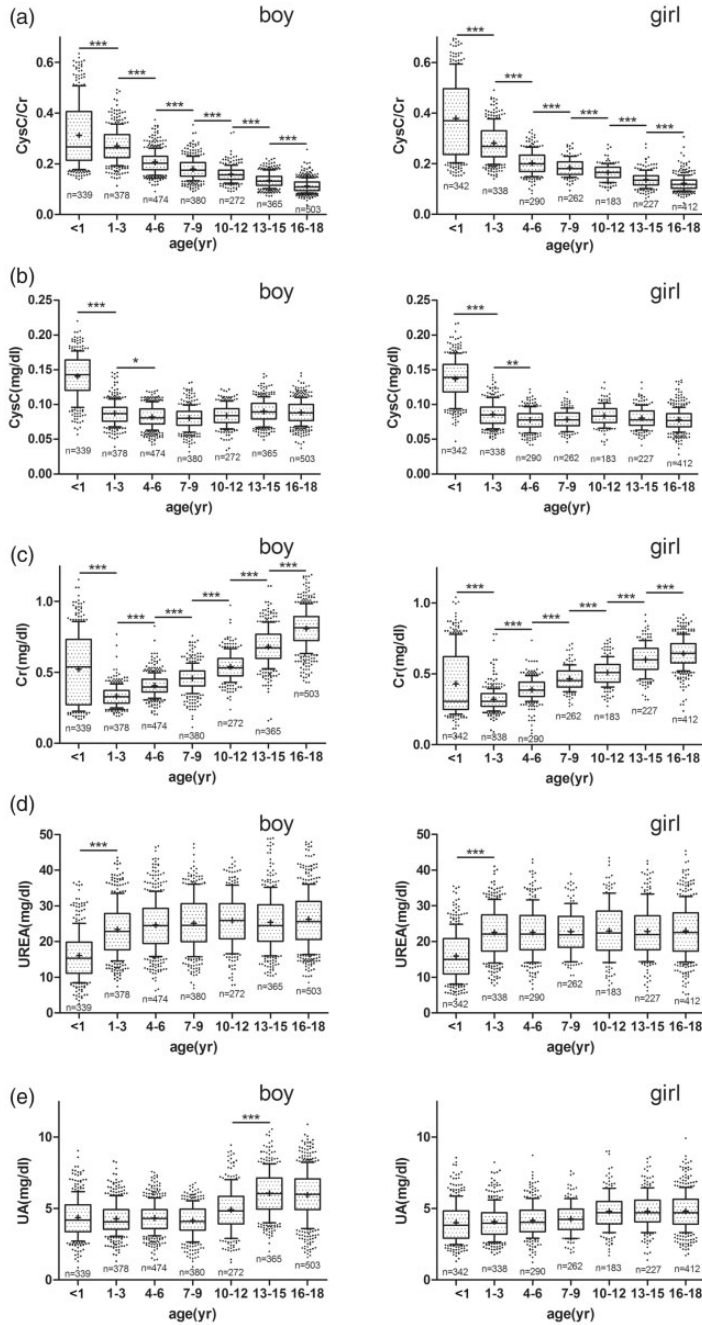


Figure 2. Box plot distributions showing serum CysC/Cr ratio (a), CysC (b), Cr (c), urea (d), and UA (e) levels (10th, 25th, 50th, 75th, and 90th centiles) for different ages and sex. +mean value, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. CysC: cystatin C; Cr: creatinine; UA: uric acid.

Table 2. Serum CysC/Cr ratio and markers of renal function by age and sex.

Age, years	Boys			Girls			P
	n	Mean	Percentile 2.5–97.5 (median)	n	Mean	Percentile 2.5–97.5 (median)	
CysC/Cr ratio							
<1	339	0.329	0.155–0.679 (0.279)	342	0.391	0.167–0.724 (0.372)	<0.001
1–3	378	0.272	0.162–0.414 (0.263)	338	0.287	0.170–0.454 (0.271)	NS
4–6	474	0.207	0.140–0.312 (0.202)	290	0.211	0.131–0.385 (0.201)	NS
7–9	380	0.181	0.111–0.279 (0.177)	262	0.192	0.110–0.257 (0.188)	0.008
10–12	272	0.159	0.105–0.227 (0.156)	183	0.170	0.108–0.294 (0.166)	0.006
13–15	365	0.137	0.086–0.202 (0.132)	227	0.156	0.082–0.200 (0.145)	0.004
16–18	503	0.112	0.071–0.170 (0.109)	412	0.134	0.079–0.207 (0.126)	<0.001
CysC (mg/dL)							
<1	339	0.140	0.076–0.197 (0.142)	342	0.138	0.083–0.196 (0.139)	0.044
1–3	378	0.087	0.059–0.126 (0.087)	338	0.089	0.058–0.150 (0.088)	NS
4–6	474	0.082	0.053–0.111 (0.081)	290	0.078	0.049–0.108 (0.078)	<0.001
7–9	380	0.083	0.049–0.120 (0.08)	262	0.079	0.053–0.105 (0.078)	<0.001
10–12	272	0.085	0.051–0.119 (0.085)	183	0.081	0.053–0.115 (0.080)	<0.001
13–15	365	0.090	0.059–0.127 (0.089)	227	0.080	0.055–0.112 (0.078)	<0.001
16–18	503	0.089	0.058–0.123 (0.088)	412	0.079	0.051–0.127 (0.077)	<0.001
Cr (mg/dL)							
<1	339	0.524	0.192–0.996 (0.538)	342	0.433	0.181–0.832 (0.306)	<0.001
1–3	378	0.331	0.226–0.469 (0.328)	338	0.322	0.204–0.555 (0.311)	0.007
4–6	474	0.406	0.272–0.577 (0.396)	290	0.389	0.196–0.532 (0.385)	0.009
7–9	380	0.459	0.260–0.674 (0.464)	262	0.466	0.318–0.668 (0.453)	NS
10–12	272	0.544	0.362–0.738 (0.532)	183	0.536	0.328–0.946 (0.521)	0.08
13–15	365	0.683	0.432–0.940 (0.674)	227	0.606	0.404–0.830 (0.600)	<0.001
16–18	503	0.810	0.498–1.113 (0.815)	412	0.655	0.423–0.879 (0.645)	<0.001
Urea (mg/dL)							
<1	339	16.400	5.048–35.850 (15.326)	342	16.174	5.658–32.594 (14.995)	NS
1–3	378	23.577	11.152–38.981 (22.838)	338	23.224	10.031–39.355 (22.297)	NS
4–6	474	24.755	11.998–41.649 (24.461)	290	22.858	10.506–39.690 (22.177)	<0.001
7–9	380	25.167	11.644–39.456 (24.521)	262	23.110	10.666–43.225 (22.147)	0.003
10–12	272	26.451	12.008–42.647 (26.023)	183	24.543	10.073–50.416 (22.568)	<0.001
13–15	365	25.711	12.212–48.869 (24.521)	227	23.503	9.581–43.576 (22.327)	0.002
16–18	503	26.472	13.372–45.706 (25.633)	412	23.234	9.792–39.947 (22.658)	<0.001
UA (mg/dL)							
<1	339	4.407	1.992–7.650 (4.203)	342	4.002	1.970–7.296 (3.825)	<0.001
1–3	378	4.354	2.320–7.185 (4.085)	338	4.102	2.186–7.040 (3.985)	0.008
4–6	474	4.356	2.400–6.849 (4.321)	290	4.207	2.107–6.878 (4.052)	0.01
7–9	380	4.160	1.836–6.225 (4.102)	262	4.362	2.206–7.440 (4.254)	NS
10–12	272	4.914	2.095–8.679 (4.825)	183	4.851	2.479–8.268 (4.724)	NS
13–15	365	6.067	3.317–9.357 (6.036)	227	4.781	1.903–7.928 (4.708)	<0.001
16–18	503	6.012	2.341–9.634 (5.985)	412	4.812	2.410–7.880 (4.708)	<0.001

P values were derived from comparison between different sexes at the same age. NS: not significant; CysC: cystatin C; Cr: creatinine; UA: uric acid.

applied for these two groups to establish reference intervals without regard to sex. The reference interval for the serum CysC/Cr ratio was 0.164 to 0.438 (median, 0.265) in children aged 1 to 3 years and 0.137 to 0.320 (median, 0.202) in children aged 4 to 6 years.

Discussion

The serum CysC/Cr ratio may be useful for diagnosing nephropathy in pediatric patients because use of serum CysC or Cr levels may not accurately assess renal impairment in children.^{9,12} Moreover, the serum CysC/Cr ratio in diagnosis of kidney disease or renal systemic disorders (e.g., postrenal acute kidney injury or shrunken pore syndrome) may be more useful than serum CysC or Cr levels.⁷⁻¹⁰ Additionally, the serum CysC/Cr ratio is expected to be an indicator for prediction or diagnosis of diseases, such as lung cancer, amyotrophic lateral sclerosis, and systemic lupus erythematosus.^{5,6,14}

The serum CysC/Cr ratio has rarely been reported in the literature. The only two available studies on reference intervals of the serum CysC/Cr ratio examined older patients and newborns.^{15,16} However, no study has reported a reference interval for healthy children (0–18 years old). In this study, we investigated the serum CysC/Cr ratio and levels of renal function indicators (CysC, Cr, urea, and UA) of 4765 healthy children. The distribution of the serum CysC/Cr ratio differed in healthy children according to age and sex, from the neonatal period to adolescence. To the best of our knowledge, this is the first study to statistically analyze and establish reference intervals for the serum CysC/Cr ratio of healthy children aged 0 to 18 years.

Consistent with the findings of Bökenkamp et al.,¹⁷ CysC and Cr levels in infants were high at < 1 year of age and then sharply decreased in our study. This finding suggested that the high serum CysC and Cr levels may have been due to

maternal origin.¹⁸ Additionally, this finding may also be due to neonatal renal insufficiency, leading to renal tubular reabsorption of CysC and Cr into the blood.¹⁹ In our study, after 1 year old, CysC levels in boys were higher than those in girls, which is consistent with the results of our previous study in adults (unpublished) and with other published reports.²⁰⁻²⁴ Cr levels showed sex differences in children starting at 10 to 12 years old. This finding is primarily because the increase in muscle mass in boys is greater than that in girls after entering puberty at the age of 10 years. This results in significantly higher serum Cr levels in boys than in girls. However, in infants younger than 1 year, Cr levels in boys were also higher than those in girls, which will require follow-up studies for explanation. Interestingly, the trend of changes in UA levels was different between sexes after 13 years old, which is consistent with previously reported results.²⁵⁻²⁸ No reasonable explanation for this phenomenon is available, but it may be related to the smaller amount of protein in the diet of adolescent girls than in adolescent boys.

Unlike the four serum biochemical markers of kidney function (CysC, Cr, urea, and UA), the serum CysC/Cr ratio gradually decreased with increasing age in healthy children from the neonatal period to adolescence. Overall, the CysC/Cr ratio in girls was higher than that of boys, which is consistent with the trend observed in the older population.¹⁵ CysC levels did not substantially change after 1 year old. Although serum CysC levels in boys were significantly higher than those in girls, the increased rate of serum Cr levels in girls was not as high as that in boys. This may be the reason that the serum CysC/Cr ratio decreased with increasing age and the serum CysC/Cr ratio was higher in girls than in boys.

There are several limitations to our study. One is the inclusion of children who were not completely healthy, especially among those

aged <1 year. Additionally, the CysC/Cr ratio of children aged <1 year was not described in detail. Therefore, further study is necessary to include healthy subjects between the ages of 0 and 1 year and to explain why there is such a large sex difference in the CysC/Cr ratio among children aged <1 year.

For the first time, this study established reference intervals for the serum CysC/Cr ratio in healthy children aged 0 to 18 years. This study shows that the serum CysC/Cr ratio is closely related to age and sex from the neonatal period to adolescence. Our findings may have some significance as a reference for diagnosis and treatment of pediatric kidney disease.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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