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Original Article

Estimating glomerular filtration rate via cystatin-C in preterm infants: a comparative analysis

Yesim Coskun[1], Ozlem Unay Demirel[2], Tevfik Bayram[3], Ipek Akman[1], Duygu Ovunç Hacihamdioglu[1]

Abstract

Background Glomerular filtration rate (GFR) is considered the best marker of renal function. Until the 36th week of gestation, GFR gradually increases due to increased size and number of nephrons. Renal clearance of inulin is known as the gold standard marker to measure eGFR. Serum cystatin-C (sCysC) is a cationic cysteine protease inhibitor produced at a constant rate from all nucleated cells and not related to infant age, gender, muscle mass, inflammation, or nutritional status. Serum creatinine (sCr) level is dependent on age, gender, maternal renal function, muscle mass, and catabolic status.

Objective To determine the course of sCr, sCysC, and urine cystatin-C (uCysC) levels as well as calculate estimated glomerular filtration rate (eGFR) using sCr- and sCysC-based formulas in preterm infants in the first 28 days of life.

Methods A total of 52 neonates were included in this prospective study. The neonates were divided into three groups according to gestational age (GA): group 1 (GA ≤28 weeks, 15 subjects), group 2 (GA 29-31 weeks, 16 subjects), and group 3 (GA 32-34 weeks, 21 subjects). Blood and urine specimens were obtained at 24-48 hours of life and then weekly until the 28th day of life. The value and course of eGFR was determined by sCr- and sCysC-based formulas.

Results The sCr level was negatively correlated with GA (r=-0.36; P=0.014), but not with BW (r=-0.15; P=0.31). While sCr levels showed significant variations in all study groups on day 7, day 14, and day 21, sCysC did not differ among groups at any time points. All study groups had significantly different uCysC levels, except at day 28.

Conclusion In preterm infants, eGFR results calculated with a sCr-based formula are detected to be closer to the inulin values. Therefore, sCr is more reliable for calculating eGFR than sCysC.

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Keywords: *estimated glomerular filtration rate; preterm infants; serum creatinine; serum cystatin-C*

Glomerular filtration rate (GFR), the flow of plasma from the glomerulus into Bowman's space per unit of time, is a dynamic variable and an easy to perform measure of renal function.[1] In utero, the GFR is slow due to the function of the placenta maintaining fluid and electrolyte balance and clearance of metabolic wastes. At birth, GFR remains low, however, it increases progressively until the end of the first month of life and reaches 20 mL/min/1.73 m^2 in both preterm and term infants.[2] Since GFR cannot be directly measured, it is evaluated by predictive formulas or by measuring clearance of an ideal filtration marker. Various endogenous and exogenous substances have been evaluated as an ideal marker for measuring GFR. Although inulin, creatinine, iohexol, ethylenediaminetetraacetic acid, diethylenetriaminepentaacetic acid, and sodium iothalamate have been used, serum creatinine (sCr)

From the Department of Pediatrics[1] and Department of Clinical Biochemistry[2], Bahcesehir University School of Medicine Goztepe Medicalpark Hospital, Department of Public Health, Marmara University School of Medicine[3], Istanbul, Turkey

Corresponding author: Yesim Coskun. Department of Pediatrics, Bahcesehir University School of Medicine Goztepe Medicalpark Hospital. Merdivenkoy, E5 uzeri, 23 Nisan Sokak, No:17, 34732 Kadikoy/Istanbul/Turkey; Telp. +90-532 255 64 79; Fax. +90-216 468 45 67; Email: coskunyesim@yahoo.com.

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is commonly used in clinical practice because it is produced at a constant rate in the human body and is predominantly removed from the body by filtration through the glomeruli.[2-6] However, in neonates, sCr has some disadvantages for measuring eGFR. First, sCr is produced from creatinine and phosphocreatine which are in muscle tissue, therefore, the sCR level is associated with muscle mass. Second, at birth, the neonatal level of sCr reflects the maternal level due to the fetomaternal-placental transition. Third, renal tubules leak in immature kidneys during passive reabsorption of filtrated creatinine. Therefore, sCR clearance can underestimate the true GFR in neonates. Fourth, sCr is not sensitive to small changes of GFR.[1,2] As a result, sCr level is an age, gender, maternal renal function, muscle mass and catabolic status dependent marker.

Cystati-C is filtered from the glomeruli, almost completely reabsorbed from the proximal tubule, and metabolized in the same zone.[7] Since it does not cross the placenta, it may more accurately reflect neonatal kidney function at birth. The sensitivity and specificity of serum cystatin-C (sCysC) for the prediction of postnatal renal function were reported to be 63.6% and 91.8%, respectively.[8] In addition, sCysC was reported to have an acceptable prognostic value to predict acute kidney injury (AKI) in children.[9] Although previous studies have reported that sCysC is a more specific and sensitive marker of GFR in children, its main disadvantages are having a wide range of sCysC levels in neonates and unknown handling of sCysC by immature neonatal kidneys.[2] On the other hand, it was argued that urinary CysC (uCysC) level, which is dependent of postnatal age and GA, may be useful to detect subclinical AKI in critically ill neonates.[10] Consequently, it is unclear which equation works best to estimate GFR in preterm infants.

The aim of this study was to determine the courses of sCr, sCysC, and uCysC levels in preterm infants in the first 28 days of life and estimate the values and courses of eGFR using five different sCr- and sCysC-based formulas.

Methods

This prospective study was conducted in Bahcesehir University School of Medicine, Medicalpark Goztepe Hospital, Neonatal Intensive Care Unit, Turkey, between November 2018 and June 2019. The study protocol was approved by the Ethics Committee of Bahcesehir University School of Medicine. Written informed consent was obtained from subjects' parents.

A total of 52 preterm infants with GA ≤34 weeks and whose serum and urine specimens had been collected during the first 4 weeks of life were enrolled. Infants who died during the study period, or were diagnosed with AKI, hemolytic anemia, thyroid dysfunction, electrolyte imbalance, or had any organ system malformations were excluded. In addition, those whose mothers had systemic illness or chronic or pregnancy-induced hypertension were excluded.

Infant GA was based on the date of the last maternal menstrual period and confirmed by the Ballard scoring system.[11] Body weight (BW) and body length (BL) were noted at birth and weekly. Transthoracic echocardiography was performed for the diagnosis of patent ductus arteriosus (PDA), which was routinely managed by ibuprofen or paracetamol. Respiratory distress syndrome (RDS) was defined as respiratory distress with characteristic radiographic results.[12] Intraventricular hemorrhage (IVH) was diagnosed by cranial ultrasonography and classified according to Papile grading system.[13] Necrotizing enterocolitis (NEC) was diagnosed by clinical and radiological results, and managed with respect to Bell's staging criteria.[14] AKI was defined according to the RIFLE (risk, injury, failure, loss of function, end-stage kidney disease) score.[15]

Blood specimens were drawn at 24-48 hours of life and then weekly (7th, 14th, 21st, and 28th days of life) in the postnatal period by venipuncture through a venous catheter at 7 am when routine blood screening tests were performed. The EDTA tubes were centrifuged for 10 minutes at 1000 g within 5 hours of collection. Measurements for sCr and sCysC plus blood urea nitrogen (BUN) were done concurrently. In addition, urine specimens were collected using urine collection bags on the same days as blood specimens. Serum and urine specimens were stored at -20°C until analysis. Serum creatinine (sCr), sCysC, and uCysC were measured by an *Abbott Architect CI8200 Autoanalyze*r (*Abbott Diagnostics*, Abbott Park, Illinois, USA). Serum creatinine (sCr) levels were measured by kinetic alkaline picrate method (0.7-1.3 mg/dL for males, 0.6-1.1 mg/dL for females). Both serum and urine CysC were measured

by immunoturbidimetric method (particle enhanced turbidimetric immunoassay) (0.57-1.09). Intra-assay coefficient of variability was 1.5% for CysC, while the inter-assay coefficient of variability was 0.5%.

Five different eGFR equations were used as follows:

1. Original Schwartz eGFRSchwartz= k× birth lenght (cm)/serum creatinine (mg/dL), where k~0.33 preterm infant.[16]
2. Dorum formula eGFRDorum= [74.835/serum cystatin $C^{1/0.75}$].[17,18]
3. Zappitelli's formula eGFRZappitelli= [75.94× (serum cystatin C)$^{-1.170}$].[19]
4. Schwartz-combined formula eGFRS-Combined= {3.98 × [birth lenght (cm)/sCr]0.456X (1.8/sCysC)$^{0.418}$ X (30/BUN)$^{0.079}$}.[20]
5. Filler formula log eGFRFiller = 1.962 + [1.123 × log(1/serum cystatin C)].[21]

For the measurement of GFR, the substance in the plasma should be in stable concentration, physiologically inert, freely filtered at the glomerulus, and is not secreted, reabsorbed, synthesized, or metabolized by the kidney. Therefore, the amount of the substance filtered at the glomerulus is equal to the amount of substance excreted in the urine. In that case, inulin clearance forms the gold standard for measuring GFR. Graphs showing the variation of eGFR curves over time were drawn for each group, using inulin as a reference. Inulin was not measured in these subjects, but taken from another study. We used inulin values determined by Brion *et al.*[22]

Statistical analyses were performed with *Statistical Package for Social Sciences* (*SPSS Inc.*, Chicago, IL, USA) software. Categorical variables were presented in frequencies. The normality of the numerical variables was examined by histograms, probability plots, and Shapiro-Wilk's test. Non-normally distributed variables were presented in medians and 25th-75th percentiles, and non-parametric tests were used for comparisons. Friedman tests were used to analyze for significant changes in GFR throughout the five postnatal time-points. Then Wilcoxon test with Bonferroni correction was used to analyze pairwise differences. Kruskal-Wallis and Mann-Whitney U tests were used to compare measurements among different GA groups. Spearman's correlation test was used for comparisons of two numerical variables. Results with P values <0.05 were considered to be statistically significant.

Results

Subjects were divided into three groups according to their GA (group 1: GA ≤28 weeks, 15 subjects; group 2: GA 29-31 weeks, 16 subjects; and group 3: GA 32-34 weeks, 21 subjects). All infants had respiratory distress syndrome (RDS). Of the 52 preterm infants, 51.9% were female and 48.1% were male. The median BW and BL of subjects at birth were 1,590 g (IQR 1,020-1,770) and 40.5 cm (IQR 37.1-43.8), respectively. In all study groups 30.8% had PDA, where 29.4% and 17.8% were treated with ibuprofen and paracetamol (seven patients using both), respectively. Detailed characteristics of the study groups are shown in **Table 1**.

At baseline, median sCr was 0.76 (IQR 0.69-0.82) mg/dL; median sCysC was 1.84 (IQR 1.61-2.11) mg/dL; and median uCysC was 0.31 (IQR 0.11-0.57) mg/dL. While sCr levels showed significant variations among study groups on postnatal day 7, day 14, and day 21, sCysC did not significantly differ at any time points. All study groups had significantly different uCysC levels at all time-points, except on day 28, when median

Table 1. Demographic characteristics of subjects by GA group

Parameters	Group 1(n=15)	Group 2 (n=16)	Group 3 (n=21)	Total (n=52)
Median body weight (IQR), g	930 (860-1,020)	1,617 (1,515-1,704)	1,780 (1,605-2,428)	1,590 (1,020-1,770)
Median birth length(IQR), cm	36 (34-38)	40 (38.5-41)	45 (42-46)	40.5 (37.1-43.8)
Female, n (%)	7 (46.7)	8 (50.0)	12 (57.1)	27 (51.9)
Patent ductus arteriosus, n (%)	9 (60.0)	3 (18.8)	4 (19.0)	16 (30.8)
Intraventricular hemorrhage, n (%)	10 (66.7)	1 (6.3)	0 (0)	11 (21.2)
Necrotizing enterocolitis, n (%)	7 (46.7)	0 (0)	1 (0.5)	8 (15.4)
Ibuprofen use, n (%)	8 (53.3)	4 (26.7)	3 (14.3)	15 (29.4) *
Paracetamol use, n (%)	5 (33.3)	1 (6.3)	3 (14.3)	9 (17.8) *

*Seven patients with PDA received both ibuprofen and paracetamol

levels were 0.00 in each group and each group had significantly reduced levels compared to that measured at baseline (group 1: P=0.003; group 2: P=0.003; and group 3: P=0.001) (**Table 2**).

The sCr level was negatively correlated with GA (r=-0.36; P=0.014), however, not with BW at birth (r=-0.15; P=0.31). Serum cysC level was not associated with either GA (r=-0.36; P=0.014) or BW at birth (r=-0.36; P=0.014). On the other hand, there was a significant inverse correlation between uCysC level and both GA (r=-0.51; P<0.001) and BW (r=-0.65; P<0.001), i.e., as GA and BW decreased, uCysC level increased.

For all GA, eGFR values that have been calculated with eGFRSchwartz formula, which includes only serum creatinine level, was found to be closest to the inulin levels in terms of absolute values and the course throughout the postnatal days. This might indicate that sCr-based formulas are more reliable than cystatin-based formulas. The weekly eGFR course and values in the post-natal period are shown in **Figure 1**.

Discussion

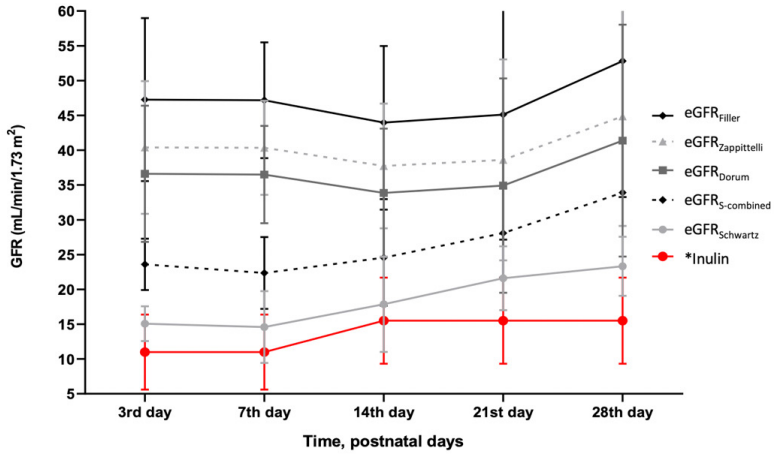
We calculated estimated glomerular filtration rate (eGFR) using sCr- and sCysC-based formulas in preterm infants in the first 28 days of life. Although previous studies have shown that sCysC is a reliable marker of renal function, only a few studies have examined consecutive measurements of sCysC levels in preterm infants, especially focusing on the first month of life.[23-27] In addition to weekly sCysC measurements, we also simultaneously examined consecutive uCysC levels.

In clinical practice, sCr level is the most used biomarker to assess neonatal renal function. In preterm infants, sCr levels are inversely related to GA and in the first few days of life, sCr levels are higher than in full term neonates. However, sCr declines gradually to the normal term reference range after the first week of life. This condition is thought to be due to immature kidney function. The sCr level is determined by renal tubule maturation, infant muscle mass, GFR, and

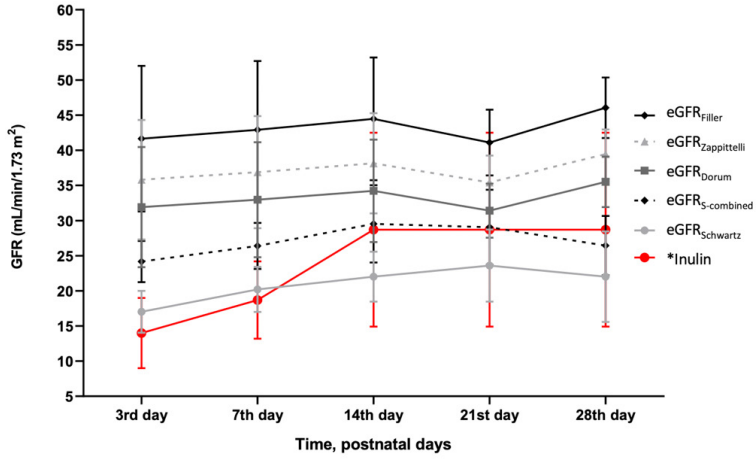
Table 2. Kruskal-Wallis analysis of sCr, sCysC, and uCysC levels among GA groups at pre-defined postnatal time points

Sampling time	GA, weeks	Serum creatinine, mg/dL		Serum cystatin C, mg/L		Urine cystatin C, mg/dL	
		Median (IQR)	P value	Median (IQR)	P value	Median (IQR)	P value
24-48 hours	≤28	0.81 (0.69-0.90)	0.078	1.70 (1.50-2.20)	0.382	0.92 (0.45-1.89)*	<0.001
	29-31	0.79 (0.74-0.82)		1.93 (1.62-2.20)		0.16 (0.09-0.46)	
	32-34	0.74 (0.68-0.79)		1.84 (1.71-2.09)		0.17 (0.08-0.31)	
	Total	0.76 (0.69-0.82)		1.84 (1.61-2.11)		0.31 (0.11-0.57)	
Day 7	≤28	0.83 (0.63-1.01)*	0.006	1.72 (1.63-1.86)	0.373	1.21 (0.52-2.04)*	<0.001
	29-31	0.65 (0.62-0.68)		1.91 (1.60-2.22)		0.16 (0.08-0.30)	
	32-34	0.60 (0.53-0.66)		1.93 (1.60-2.08)		0.09 (0.04-0.16)	
	Total	0.65 (0.58-0.80		1.86 (1.62-2.08)		0.19 (0.07-0.75)	
Day 14	≤28	0.68 (0.57-0.92)§	0.044	1.95 (1.59-2.06)	0.633	1.26 (0.47-1.94)*	<0.001
	29-31	0.59 (0.55-0.63)*		1.86 (1.56-2.06)		0.24 (0.05-0.84)*	
	32-34	0.54 (0.48-0.58		1.85 (1.73-2.22)		0.04 (0.04-0.15)	
	Total	0.58 (0.52-0.68)		1.87 (1.65-2.13)		0.17 (0.04-0.98)	
Day 21	≤28	0.54 (0.48-0.66)§	0.049	1.84 (1.74-2.09)	0.856	0.20 (0.07-0.80)§	0.024
	29-31	0.53 (0.50-0.61)		2.00 (1.81-2.06)		0.10 (0.06-0.30)	
	32-34	0.44 (0.40-0.53)		1.89 (1.74-2.03)		0.06 (0.04-0.13)	
	Total	0.50 (0.45-0.61)		1.88 (1.77-2.07)		0.08 (0.04-0.26)	
Day 28	≤28	0.52 (0.44-0.63)	0.244	1.76 (1.35-2.00)	0.205	0.00 (0.00-0.18)**	0.604
	29-31	0.63 (0.46-0.76)		1.79 (1.67-1.85)		0.00 (0.00-0.04)**	
	32-34	0.53 (0.43-0.60)		2.04 (1.80-2.18)		0.00 (0.00-0.05)**	
	Total	0.54 (0.44-0.63)		1.90 (1.58-2.10)		0.00 (0.00-0.07)	

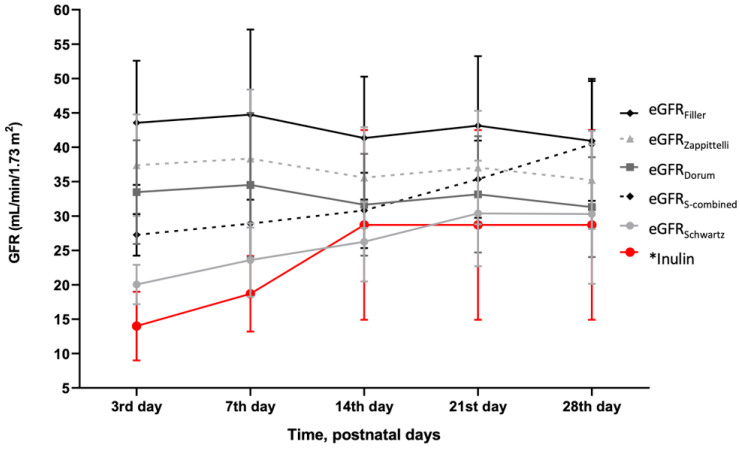
*P<0.05 *vs.* both other groups in post-hoc tests; §P<0.05 *vs.* GA 32-34 weeks (group 3) in post-hoc tests; **P<0.05 *vs.* 24-48 hours; GA= gestational age; IQR=interquartile range



(A) Group 1: GA <28 weeks



(B) Group 2: GA 29-31 weeks



(C) Group 3: GA 32-24 weeks

Figure 1. The value and course of eGFR according to the five formulas and inulin [* The reference values determined by Brion *et al.*[22]]

tubular secretion.[2,28-30] Similarly, we found that sCr levels of preterm infants were associated with GA and postnatal age. The sCr levels decreased in the 3rd week of life to a mean value of 0.50 mg/dL in all study groups compared to baseline sCr level of 0.76 mg/dL. According to the weekly sCr analysis, a statistically significant decrease was found which was compatible with the literature.[2,5,6,30-32]

Despite the widespread use of sCr to assess renal function, markers which are stable over time and not affected by tubular reabsorption or secretion, as well as other factors such as muscle mass, BW or nutritional status would be more accurate. The sCysC marker has attracted attention as it is thought to be a reliable marker of renal function and more useful than sCr for detecting renal function in critically ill children.[33-36] Several studies have evaluated sCysC for calculating eGFR in preterm infants. A prospective study in preterm infants who had uncomplicated clinical courses found that sCysC was a better marker than sCr for estimating GFR in the first year of life.[37] A study in preterm infants (GA 27-32 weeks) with and without RDS to detect AKI and concluded that sCysC level was significantly associated with the prediction of AKI development.[38] In addition, another study of preterm infants <34 weeks' gestation with RDS demonstrated that sCysC measured postnatally on the 3rd day of life could predict AKI earlier than sCr.[39] Moreover, Nakashima *et al.*[37] showed that sCysC was more reliable than sCr for calculating eGFR in preterm infants. Other studies were done to assess the accuracy of sCysC for estimating GFR in the postnatal period. A longitudinal study done on 21 healthy neonates demonstrated that sCysC levels decreased from day 0 to day 3 after birth and afterwards remained constant up to 1 month of life. Moreover, neonatal sCysC levels were not affected by maternal sCysC levels.[40] A study on neonatal sCysC reference levels, including very low birth weight infants, showed that except in the first 3 days of life, sCysC levels decreased gradually as the post-conceptional age increased.[41] Similarly, a previous study in 108 infants with mean GA 32.5 (SD 2.6) weeks and found that day 1 postnatally, sCysC concentrations were high, however, they decreased by day 3.[42] According to a prospective study in preterm neonates (GA 28-34 weeks), sCysC levels were high postnatally on day 3, and decreased by day 30.[43] The authors concluded that the decrease in sCysC levels in the neonatal period may have been due to maturation of renal function. In contrast, we found that sCr was more reliable than sCysC for estimating GFR. Moreover, we did not find any statistically significant correlation with sCysC levels with regards to postnatal days, GA, or BW.

Although the course of sCysC during severe diseases remains unclear, it has been argued that sCysC does not reliably reflect renal function of neonates with sepsis.[44] Furthermore, asphyxiated neonates had significantly higher sCysC levels in the first days of life compared to healthy ones.[45] In our study, the preterm infants with additional problems, such as IVH and NEC plus ibuprofen use, were not excluded because preterm infants frequently have serious complications and it was difficult to find a preterm subject with no complications. Therefore, in order to use sCysC to evaluate the accuracy of renal function among preterm infants with severe medical problems, more studies are needed with a focus on factors affecting sCysC metabolism in the neonatal period.

As shown in **Table 2**, uCysC levels had a negative correlation with postnatal age and GA. In group 1, uCysC levels were significantly higher than that of the other two groups on most postnatal days except for day 28. The decrease in uCysC levels, especially in group 1, reflects progressive glomerular recruitment. Tubular immaturity leading to tubular leakage may cause this condition. In our subjects, tubular CysC metabolism in preterm infants ≤28 weeks' gestation was not comparable with the other two groups until the 28th day of life postnatally.

Renal clearance of inulin during intravenous administration still remains the gold standard to determine eGFR.[27] The diagnostic accuracy of eGFR varies with different CysC equations.[46] In our study, the highest eGFR course was found with the Filler equation. For groups 1 and 3 in the first 28 days of life, the value and course of eGFR calculated with the original Schwartz equation was more reliable than other equations. For group 2, in the first week of life the original Schwartz equation and after the 14th day of life the Schwartz-combined formula, were closer to the values and course of inulin. Thus, in our study, the SCr-based formula was closer to GFR values of previous report.[22]

Our study had some limitations including the

small sample size and difficulty finding healthy preterm infants because most preterm infants, especially those with <32 weeks' gestation, have various medical problems. In addition, infants whose PDA was treated with ibuprofen or paracetamol but did not develop AKI were also included. Furthermore, we did not have a gold standard measure of eGFR, such as inulin-based eGFR, with which to compare our results. Instead, we used historical data for inulin clearance from a previous study, albeit from a premature infant population with similar BW and BL values.[22] Besides,the Jaffe and turbidimetric methods we used for measuring sCr and sCysC, respectively, might have led to an overestimation of eGFR values in our patient population, for whom enzymatic and nephelometric methods were recommended, respectively.[47]

In conclusion, in preterm infants, eGFR values calculated with sCr-based formulas were found to be closer to inulin clearance in the first 28 days of life, indicating that sCr is more reliable than sCysC. Moreover, different factors may affect sCysC levels in premature infants other than previously reported ones. Since organogenesis is expected to continue in the postnatal period even if the infant is in an impaired environment, uCysC levels may reflect this maturation more than sCysC. Further studies are needed with large sample sizes highlighting factors which may affect the course and fluctuations of sCysC levels in preterm infants.

Conflict of Interest

None declared.

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