ORIGINAL ARTICLE

Estimated glomerular filtration rate in apparently healthy term neonates in Nigeria

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ABSTRACT

Background: Glomerular filtration rate (GFR) is the best measure of renal function. However, the ideal exogenous markers are not routinely used to estimate GFR in clinical settings. Outside Africa, GFR estimates for neonates have been calculated from formulae using creatinine as well as cystatin C. There is a paucity of data on estimated GFR in neonates in Africa. This study was conducted to estimate GFR in healthy term African neonates using cystatin C-based equations.

Methods: One hundred and thirteen apparently healthy term babies with normal Apgar scores were recruited at birth. Cystatin C was measured in cord blood at birth and in venous samples from neonates on the third day of life. GFR was estimated using the Filler and Lepage as well as the Zappitelli et al. cystatin C-based equations. These estimates were compared with a reference GFR estimate of 41 ± 15 mL/min/1.73 m^2.

Results: The median (interquartile range) estimated GFR values based on cord blood and day 3 venous samples using the Filler and Lepage equation were 44.67 (36.35–72.44) and 51.29 (38.90–72.44) mL/min/1.73 m^2, respectively. When the Zappitelli equation was applied, the estimates were 38.18 (31.84–61.34) and 43.82 (33.75–61.34) mL/min/1.73 m^2, respectively. The GFR estimates derived from both equations did not differ in respect of the gestational age, sex and postnatal ages of the babies.

Conclusions: GFR estimates using the Zappitelli equation had values that were closely related to reference GFR measures for neonates whereas the GFR estimates using the Filler and Lepage equation had higher values.

Keywords: GFR, neonates, cystatin C, cord blood.

INTRODUCTION

Glomerular filtration rate (GFR) is defined as the volume of plasma that is completely cleared of a particular substance by the kidneys in a unit of time [1]. GFR is widely accepted as the best measure of renal function [2-4]. In order to detect abnormal renal function, it is important to establish reference values for GFR using reliable methods of evaluation.

The most precise method for measuring GFR is to use exogenous markers like inulin. Measurement of inulin clearance involves the intravenous injection of a priming dose followed by constant infusion and meticulous urine sampling. Other exogenous markers such as iohexol, sodium iothalamate, technetium-diethylenetriamine-pentaacetic acid (Tc-DTPA), and chromiume-thylene-diaminetetraacetic acid (Cr- EDTA) have also been used. Although the use of these exogenous markers is the gold standard, the need for frequent blood sampling, careful urine collection and constant infusions limits their use in the clinical setting. Because of these limitations, re-searchers have sought simpler methods of assessing GFR.

Serum creatinine has been used to estimate GFR for decades. Plasma or serum creatinine as a marker of renal

function is not ideal as it is dependent on muscle mass, gender, age and tubular maturity [5,6], it crosses the placenta and thus reflects maternal values in the first days of life [6,7] and it is insensitive to small changes in GFR [6]. Finally, the Jaffe method, which is the most commonly used method for measuring creatinine, is prone to interference in conditions such as hyperbilirubinaemia, haemolysis and ketosis [1,6]. This leads to falsely high serum creatinine measurements and underestimation of GFR.

Serum cystatin C has been proposed as a more reliable marker in children and adults and offers significant advantages over serum creatinine. Cystatin C may also be used to replace creatinine when assessing renal function in neonates. Cystatin C is a cysteine proteinase inhibitor, which is involved in the intracellular catabolism of proteins [6]. It is expressed in all nucleated cells and produced at a constant rate [8]. Many studies have validated the use of cystatin C as a marker of renal function in paediatric populations [1,9,10]. Cystatin C appears to be a more specific and sensitive marker of GFR in neonates and older children compared to creatinine [1,10,11-14].

There are few studies that have evaluated renal function in neonates using ideal exogenous markers [15]. In studies conducted outside of Africa, GFR estimates have been reported using the clearance of creatinine [16,17] and cystatin C [15,18]. To the best of our knowledge, no GFR estimates have been documented in African neonates. The objectives of the present study were to estimate GFR in apparently healthy neonates in Nigeria at birth and on the third day of life, using cystatin C-based equations, and to compare the estimated GFR with previously published reference values [15,16]. This study therefore provides useful data on the normal values for renal function in healthy African neonates. The results reported here focus on GFR and represent part of a larger study; details of the methods and results of cystatin C levels have been published previously [22].

METHODS

This was a prospective study of apparently healthy full-term neonates delivered at the Obstetrics and Gynaecology unit of a Mother and Child Health Facility in Lagos State from October to December 2014. The hospital is the Obstetrics and Gynaecology annex of the Lagos State University Teaching Hospital (LASUTH), a tertiary institution in South West Nigeria.

We recruited 113 full-term, apparently healthy babies after normal pregnancy, labour and delivery. These were babies with a 5-minute Apgar score of more than 7, and who had a birth weight that was appropriate for gestational age. Babies who experienced conditions likely to affect renal function were excluded. These included babies of mothers

with hypertension, babies with gross congenital anomalies and those who developed fever or evidence of systemic illness by the third day of life.

Blood samples were obtained from the umbilical cord at birth and venous samples on the third day of life. Serum cystatin C was measured on the samples using Human Cystatin C ELISA kits (BioVendor-Laboratorni a.s., Czech Republic) [19]. The calibration range of the assay was 200–10,000 ng/mL, with a limit of detection of 0.25 ng/mL. Its intra-assay coefficient of variation was 2.5% and the inter-assay coefficient of variation was 6.9%.

Estimated GFR (eGFR), expressed in mL/min/1.73 m^2, was calculated using two prediction equations, with cystatin C concentration in mg/L:

Filler and Lepage equation [20]: Log(eGFR) = 1.962 + [1.123 × log (1/cystatin C)]

Zappitelli et al. equation [21]: eGFR = 75.94 × cystatin C $^{-1.17}$.

Data analysis was preformed using Microsoft Excel 2013 and the Statistical Package for Social Sciences (SPSS) version 20.0. Mean and standard deviation were calculated for variables that were normally distributed; median and interquartile range were used for variables that were not normally distributed. Comparisons between groups were made using Student's t-test, and the Wilcoxon signed-rank, Mann–Whitney U and Kruskal–Wallis tests were used for non-parametric data. P-values of less than 0.05 were considered statistically significant.

Approval for the study was granted by the Health Research and Ethics Committee of LASUTH. Informed consent was obtained from the parents of the neonates. The parents of babies with congenital anomalies or evidence of renal impairment were counselled and referred for appropriate follow-up.

RESULTS

A total of 144 babies were recruited, but 24 did not return for evaluation on the third day of life, or were ill on that day, and so were excluded from the study. Another seven had haemolysed samples and were also excluded. The remaining neonates were 45 males and 68 females (ratio 1:1.5). Their baseline characteristics are summarised in Table 1. Most of the babies were delivered at 37–38 weeks. The birth weight ranged from 2.5–4.5 kg, with a mean weight of 3.4 ± 0.5 kg.

The mean cystatin C values at birth and on day 3, as reported previously, were 1.67 ± 0.52 mg/L and 1.62 ± 0.52 mg/L, respectively, with medians of 1.8 and 1.6 mg/L [22]. There were no differences based on the time of sampling or the sex of the neonates (Figure 1).

Table 1. Baseline characteristics of the participants.

Variable	Frequency	Percentage
Sex		
Male	45	40.7
Female	68	59.3
Gestational age at birth (weeks)		
37–38	57	50.4
39–40	42	37.2
≥41	14	12.4
Weight at birth (g)		
2500–2999	28	24.8
3000–3499	46	40.7
≥3500	39	34.5
Mode of delivery		
SVD*	30	26.5
Caesarean section	83	73.5
Maternal age (years)		
≤18	0	0
19–30	49	45
31–40	58	53.2
≥41	2	1.8

*SVD, spontaneous vaginal delivery.

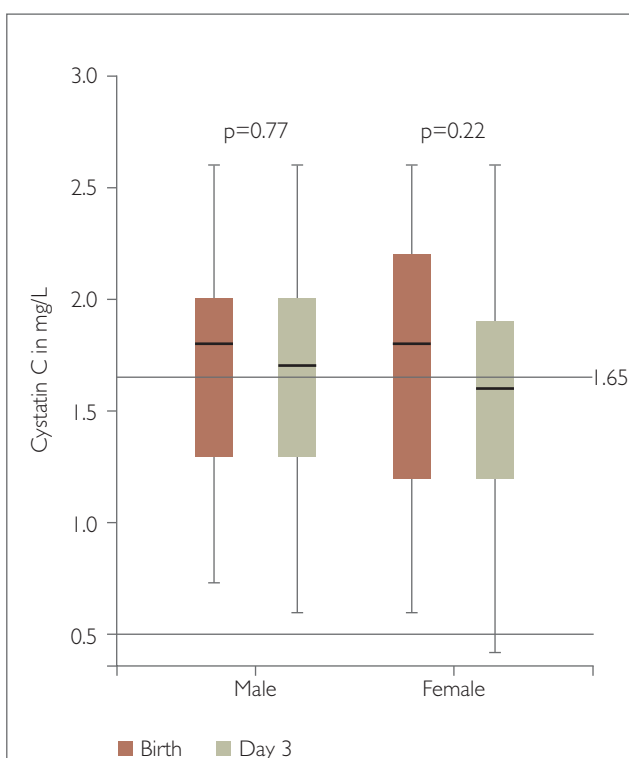**Figure 1.** Serum cystatin C in samples from neonates at birth and on day 3. From Madise-Wobo et al. [22].

Table 2. Estimated GFR* (median and interquartile range) using the Filler and Lepage equation [20].

Gender	n	Birth	Day 3	p
Male	45	44.67 (34.68–76.82)	53.70 (38.90–72.44)	0.34
Female	68	44.47 (38.02–70.85)	49.58 (41.69-67.23)	0.98
Total	113	44.67 (36.35–72.44)	**51.29 (38.90–72.44)**	**0.53**
p		0.69	0.57	

*GFR in mL/min/1.73 m².

Table 3. Distribution of eGFR values using the Filler and Lepage [20] equation at birth and on day 3.

GFR (mL/min/1.73 m²)	Birth [n=113 (%)]	Day 3 [n=113 (%)]
≤25.0	0 (0.0)	0 (0.0)
25.1–40.0	41(36.3)	30 (26.5)
40.1–55.0	25 (22.1)	33 (29.2)
55.1–70.0	18 (15.9)	20 (17.7)
≥70.1	29 (25.7)	30 (26.5)

Table 4. Effect of gestational age on eGFR values using the Filler and Lepage equation at birth and on day 3.

Age (weeks)	n	Birth	Day 3	p
37	18	55.07	58.94	0.796
38	39	47.35	50.50	0.766
39	16	62.78	58.42	0.887
40	26	47.35	59.60	0.192
41	14	44.56	46.6	0.638
p		0.425	0.454	

Table 2 shows the eGFR values calculated using the Filler and Lepage equation [20]. The median eGFR was 44.67 and 51.29 mL/min/1.73 m² at birth and on day 3, respectively. There was no difference between males and females or between the samples taken at birth and on day 3.

The eGFR values using the Filler and Lepage equation were grouped from the lowest to the highest (Table 3). There were no values less than 25 mL/min/1.73 m². The distribution of eGFR values was similar for both birth and day 3 samples (p = 0.403).

There was no significant difference in the eGFR values across the different gestational ages for both cord blood and day 3 venous samples (Table 4).

The eGFR values calculated using the Zappitelli equation [21] are shown in Table 5. Once again, there was no difference between males and females or between the samples taken at birth and on day 3.

The distribution of eGFR values using the Zappitelli equation (Table 6) was similar for samples taken at birth and on day 3. The largest number of samples was in the group with eGFR 25.1–40 mL/min/1.73 m².

As noted with eGFR values calculated using the Filler and Lepage equation, there was no difference by gestational age at birth or by day 3.

The eGFR values obtained using the Filler and Lepage equation were significantly higher than those using the Zappitelli equation (Table 7, p < 0.001).

Figure 2 illustrates the distribution of eGFR values derived from both equations. The plot depicts medians and interquartile ranges, and outliers; the reference GFR is represented by the horizontal line across the graph. The spread of the estimates from the Filler and Lepage equation was greater and the estimates from the Zappitelli equation
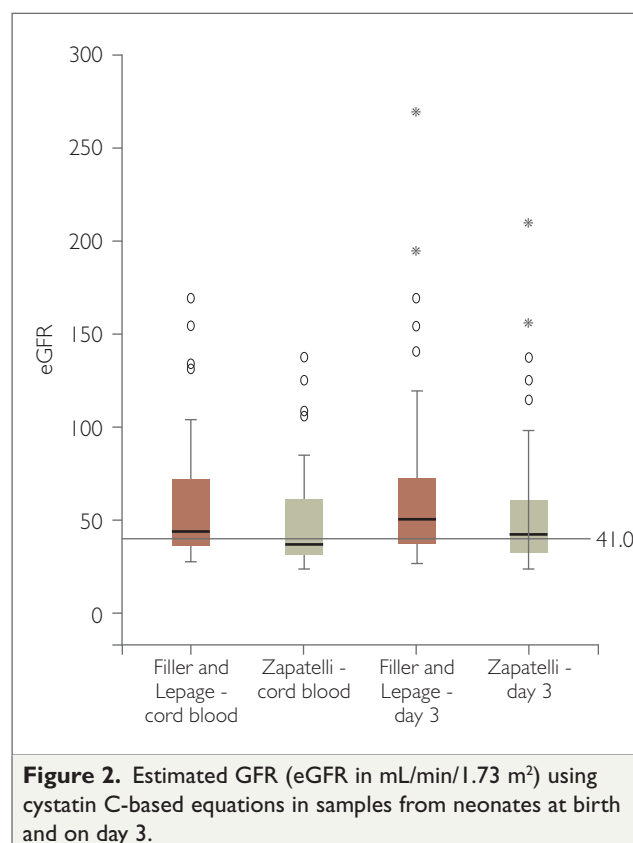

Figure 2. Estimated GFR (eGFR in mL/min/1.73 m²) using cystatin C-based equations in samples from neonates at birth and on day 3.

Table 5. Estimated GFR values using the Zappitelli equation.

Gender	eGFR median and interquartile range (25th and 75th percentiles)		
	Birth	Day 3	p
Male	38.18 (30.18–64.63)	45.83 (33.75–61.34)	0.39
Female	38.18 (33.75–59.98)	42.32 (35.84–56.63)	0.98
Total	38.18 (31.84–61.34)	43.82 (33.75–61.34)	0.60
p	0.93	0.34	

Table 6. Distribution of eGFR values using the Zappitelli equation at birth and on day 3.

GFR (mL/min/1.73 m²)	Birth [n (%)]	Day 3 [n (%)]	P
≤25.0	5 (4.4)	4 (3.5)	0.807
25.1–40.0	54 (47.8)	47 (41.6)	
40.1–55.0	19 (16.8)	24 (21.2)	
55.1–70.0	18 (16.0)	22 (19.5)	
≥70.1	17 (15.0)	16 (14.2)	

Table 7. Comparison of eGFR values using the Filler and Lepage, and Zappitelli equations.

	Filler and Lepage	Zappitelli	p
≤25.0	44.67 (36.35–72.44)	38.18 (31.84–61.34)	0.002
≥70.1	51.29 (38.90–72.44)	43.82 (33.75–61.34)	0.003

were more closely related to the reference value for both birth (cord blood) and day 3 samples.

DISCUSSION

We found that eGFR values calculated using the Filler and Lepage equation were higher than the reference values obtained from creatinine clearance in previous studies [17,23]. The eGFR values were also higher than those reported by Treiber et al. using a cystatin C-based equation formulated for neonates [15]. One reason for these differences may be that the Filler and Lepage equation overestimates GFR in children [13,15,24,25]. Since the development of the cystatin C equation by Filler and Lepage, some researchers have noted that the equation overestimates GFR especially in the low GFR ranges [15,26], whereas others reported that the equation correctly estimates GFR in children [21].

GFR was also estimated using the Zappitelli equation [21] because it has been proven to be more reliable when applied to older children and neonates [15,18,26,27]. eGFR derived from the Zappitelli equation in our study was comparable with the measured reference range from creatinine clearance [17,23] and the eGFR from the recently published cystatin C equation derived for neonates [15]. Other researchers have also estimated GFR in neonates using the Zappitelli equation in neonates and reported that the eGFR from that equation was comparable with reference values measured by creatinine clearance [15,18,27].

In the present study, eGFR values calculated using the Filler and Lepage equation were significantly higher than those from the Zappitelli equation. This finding has also been documented in the study by Treiber et al. [15]. Given that the GFR estimates from the Zappitelli equation were more closely related to the reference GFR from the literature, the estimates from that equation may be used as reference values when investigating neonates at risk of renal injury in the first few days of life.

We observed that eGFR was independent of the gestational age, postnatal age and sex of the babies. This was not surprising, given that, in utero, GFR plateaus after the 36th week of gestation until birth. Thereafter, GFR correlates with postnatal age [6,23]. Previous reports have shown that there is no significant difference in the values in the first three days of life [15,28]. The implication is that separate reference values need not be generated for sex, gestational age of term babies and postnatal age in the first three days of life.

Some limitations were encountered: first, the inability to determine GFR using a gold standard method, which would have been compared with the estimates calculated from the cystatin C equations. Second, the study included only term babies sampled at birth and on the third day of life. Third, the study would have added more value if preterm babies had been included and if all babies were also sampled at one month of age. The cost of the assays restricted the scope of the study.

CONCLUSIONS

GFR calculated using the Filler and Lepage equation yielded higher values than the estimates derived from the Zappitelli equation, which were comparable with GFR estimated using creatinine clearance in neonates.

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