Original Article

Female preterm indigenous Australian infants have lower renal volumes than males: A predisposing factor for end-stage renal disease?

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SUMMARY AT A GLANCE

Indigenous Australians are known to have an increased risk of developing CKD. The authors examined total renal volume (TRV) in a cohort of Indigenous and non-Indigenous preterm neonates, and found that female Indigenous neonates had significantly smaller TRV compared with Indigenous male neonates.

ABSTRACT:

Aim: **Indigenous Australians have an increased risk of developing chronic kidney disease (CKD). Indigenous women have a higher rate of CKD than men. In a cohort of Indigenous and non-Indigenous preterm neonates, we assessed total renal volume (TRV) (a proxy indicator for nephron number). We hypothesized that there would be no difference in renal volume between these two groups at term corrected (37 weeks gestation).**

Methods: **Normally grown preterm neonates less than 32 weeks of gestation were recruited and at term corrected dates, the neonates underwent renal ultrasonography (TRV measurements), urine microalbumin-creatinine ratio and serum analysis for Cystatin C measurement for estimated glomerular filtration rate (eGFR) calculation.**

Results: **One hundred and five neonates (38 Indigenous; 67 non-Indigenous) were recruited. Indigenous neonates were significantly more premature and of lower birth weight. At term corrected age, Indigenous neonates had a significantly smaller TRV (18.5 (4.2) *vs* 21.4 (5.1) cm^3; *P* = 0.027) despite no significant difference in body weight. Despite having a smaller TRV, there was no significant difference in eGFR between Indigenous and Non-indigenous neonates (47.8 [43.2–50.4] *vs* 46.2 [42.6–53.3] ml/min per 1.73 m^2; *P* = 0.986). These infants achieve similar eGFR through hyperfiltration, which likely increases their future risk of CKD. There was no difference in microalbumin-creatinine ratio. Female Indigenous neonates, however, had significantly smaller TRV compared with Indigenous male neonates (15.9 (3.6) *vs* 20.6 (3.6) cm^3; *P* = 0.006), despite no difference in eGFR, birth weight, gestational age, and weight at term corrected.**

Conclusion: **The difference in TRV is likely to be an important risk factor for the difference in morbidity and mortality from renal disease reported between male and female Indigenous adults.**

Australia's Aboriginal and Torres Strait Islander people make up approximately 2.8% of the Australian population.[1] This group, however, experiences a disproportionately higher prevalence of morbidity and mortality compared with the non-Indigenous Australian population. Aboriginal and Torres Strait Islander women generally have more children at a younger age compared with non-Indigenous women. The mean birth weight of babies born to Indigenous mothers is 3215 g, 140 g less than non-Indigenous neonates

and they are almost twice as likely to be of low birthweight (less than 2500 g) or premature.[2] The most recent estimates indicate that an Indigenous male born between 2010 and 2012 can be expected to have a life expectancy of 69.1 years, approximately a decade less than a non-Indigenous male (79.7 years). An Indigenous female born in 2010–2012 is expected to have a life expectancy of 74 years, also approximately a decade less than a non-Indigenous female.[3] Data indicate that almost one in ten Aboriginal and

Torres Strait Islander adults have diabetes mellitus, two in three (65.3%) have at least one risk factor for cardiovascular disease, and nearly one in five (17.9%) showed signs of chronic kidney disease (CKD).[4]

Renal disease is a major health challenge for Aboriginal and Torres Strait Islander people. In 2015, end-stage renal disease and the need for renal replacement therapy was nearly five times higher in Aboriginal and Torres Strait Islander people.[5] The major contributor to this disproportionately higher prevalence of renal disease is likely to be a combination of modifiable risk factors, including foetal growth restriction and low birth weight, prematurity, poor nutrition in infancy, childhood and adult life, elevated body mass index, diabetes mellitus, infections and inflammation.[6] While, in non-Indigenous Australians, males experience an excess of renal failure at a younger age, in the Indigenous community it is females who experience renal failure more commonly and often at a young age. This is important as this places young Indigenous mothers at risk of renal impairment which may have a profound effect on their foetuses' renal development and function.[7]

The human kidney develops from early gestation, with the mesonephros and metanephros developing at about 5 weeks.[8] The metanephros (which functions as the adult kidney) continues to grow with an increasing number of nephrons until 34–36 weeks of gestation when nephrogenesis is complete. Nephrogenesis starts in the juxtamedullary region and then progresses in an outward direction.[9] The renin-angiotensin-aldosterone system and vascular endothelial growth factor have crucial roles in nephrogenesis.[10–12] While histopathological examination can determine nephron number,[13] this is not feasible for neonates. Renal volume measurements by ultrasonography have a good correlation with nephron number[14,15] and can be used as a surrogate index of nephron number.

We carried out a study to compare renal volumes (and hence nephron numbers N_{Glom}) between a cohort of Indigenous and non-Indigenous premature neonates. We hypothesized that there would be no difference in renal volumes (and hence nephron) number between these two groups.

METHODS

Study population

This prospective study was carried out at the Department of Neonatology, The Townsville Hospital, Queensland, Australia. The study period was from August 2010 until October 2016, and the region has approximately 10 000 births per year. Preterm neonates less than 32 weeks of gestation, with average birth weights (weight between the 10th and 90th centile), were eligible to participate in this study. Neonates with growth restriction, or large for gestational age, or with congenital abnormalities were excluded. Once recruited, study subjects were followed until discharge and assessment were carried out at term corrected (37 weeks postmenstrual age (PMA)). PMA for the preterm neonates was defined as follows: PMA = gestational age at birth (weeks) + postnatal age in weeks.[16] The Townsville Health District Human Research Ethics Committee approved this study. Written consent was obtained from parents of all participants.

Data collection

At term corrected, the neonates underwent renal ultrasonography, urine analysis for the microalbumin-creatinine ratio (ACR) and venepuncture for cystatin C (CysC) measurements.

Cystatin C measurement

We used CysC as it is a more reliable marker to assess renal function in neonates.[17,18] Serum CysC measurements were carried out using an immunoturbidimetric assay designed for use on the Beckman AU480 automated analyser platform (Gentian, Australia). The assay range is 0.34–7.95 mg/L, with an intra and inter-assay precision <10%. The Zappitelli CysC estimated glomerular filtration rate (eGFR) equation was used to calculate eGFR (GFR (mL/min per 1.73 m^2) = 75.94/[serum CysC$^{1.17}$]).[19]

Urine analysis for albumin

Urine ACR measurement was carried out on a randomly collected specimen, using the Beckman Coulter microalbumin immunoturbidimetric assay on an automated Unicel DxC analyser (Beckman Coulter, Australia). The assay was calibrated using the SYNCHRON Systems MA calibrator specifically for urine assays (Beckman Australia), traceable to the International Federation of Clinical Chemistry reference range.

Renal ultrasonography

Renal ultrasonography was performed using the Philips IU22 Ultrasound System (Philips Healthcare, Andover, MA, USA) with a compact (small footprint) curved linear 5–8 MHz frequency transducer. The renal scans were carried out by the same sonographer, with an intra-class coefficient for the intra-observer variability of 0.85 (95% CI = 0.73–0.91). In both kidneys, renal length (L), antero-posterior diameter (AP), and transverse diameter (W) were measured and renal volume (KV; cm^3) was calculated using the formula: KV = (π/6 × L × W × AP).[20] The total renal volume (TKV) (Right KV + Left KV) was also calculated.
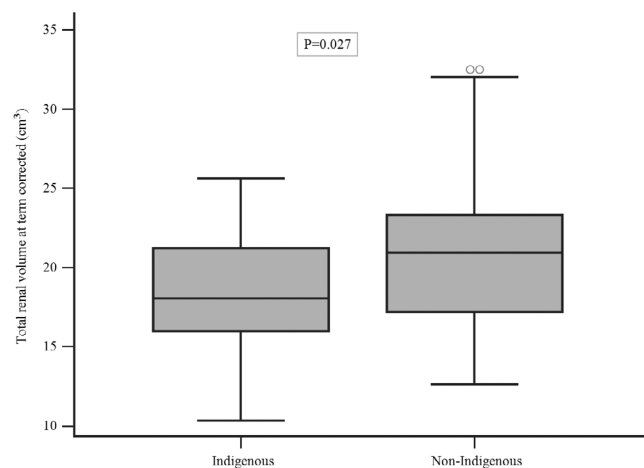
Statistical analysis

The variables are expressed as means (standard deviation (SD)) for continuous, normally distributed data and as

median [Interquartile range [IQR]] for continuous, non-normally distributed data. Students *t*-test or Mann–Whitney *U* test was used where appropriate and a *P* value <0.05 was considered statistically significant. D'Agostino-Pearson test[21] was utilized to determine the normality of the variables and statistical analyses were performed using MedCalc for Windows, version 16.4.3 (MedCalc Software, Ostend, Belgium).
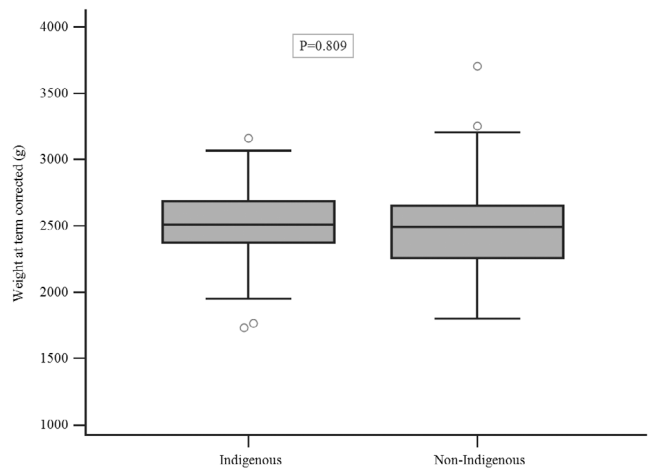
RESULTS

A total of 375 preterm neonates who fulfilled the inclusion criteria were admitted during the study period, and there were 33 deaths. One hundred eight of the available 345 neonates were recruited, and a complete data set was available for 105 neonates (38 Indigenous and 67 non-Indigenous). The median birth weight was 908 [779–1158] g, and the mean gestational age was 27.0 (2.1) weeks. There was an almost equal number of male (*N* = 53) and female (*N* = 52) neonates. There was no significant difference in the gestation (27.0 (2.4) *vs* 27.0 (1.8) weeks; *P* = 0.980) and birth weight 920 [798–1320] *vs* 892 [770–1035] g; *P* = 0.062) between male and female neonates. At term corrected gestation age, there were no significant differences in weight (2566 (371) *vs* 2416 (382) g; *P* = 0.088) or TRV (21.4 (4.4) *vs* 19.6 (5.5) cm³; *P* = 0.110) between male and female neonates.

Indigenous neonates were more premature (26.1 (1.7) *vs* 27.5 (2.2) g; *P* = 0.001) and of lower birth weight (830 [730–995] *vs* 970 [800–1274] g; *P* = 0.020). At term corrected age, Indigenous preterm neonates had significantly smaller TRV (18.5 (4.2) *vs* 21.4 (5.1) cm³; *P* = 0.027) despite no significant differences in body weight (2476 (373) *vs* 2499 (389) g; *P* = 0.809) (Figs. 1 and 2, respectively). Despite having smaller TRV at term corrected age, there was no significant difference in eGFR between Indigenous and non-Indigenous preterm neonates (47.8
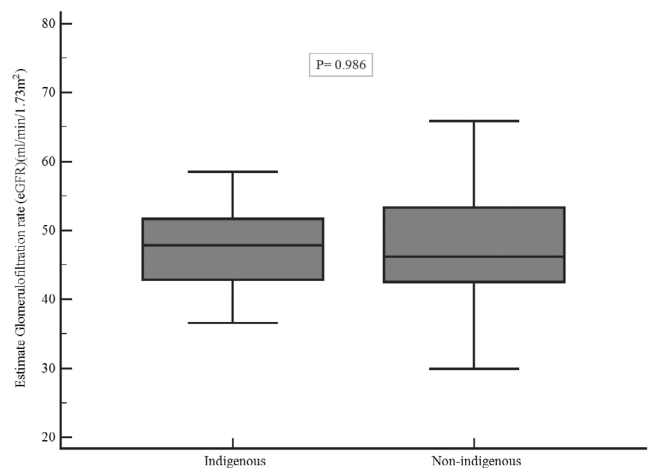
[43.2–50.4] *vs* 46.2 [42.6–53.3] ml/min per 1.73 m²; *P* = 0.986) (Fig. 3). There was no difference in urine ACR between Indigenous and non-Indigenous neonates (10.0 [6.2–28.8] *vs* 8.2 [5.0–17.0]; *P* = 0.312).

Female Indigenous preterm neonates had significantly smaller TRV when compared with Indigenous male neonates (15.9 (3.6) *vs* 20.6 (3.6) mL; *P* = 0.006, Fig. 4). There was no significant difference in eGFR (51.6 [37.2–65.8] *vs* 44.8 [36.5–74.2]] mL/min per 1.73 m²; *P* = 0.307), birth weight (890 (201) *vs* 913 (249) g; *P* = 0.771), gestational age (26.7(1.6) *vs* 25.8(1.7) weeks; *P* = 0.110) or weight at term corrected (2353 (389) *vs* 2573 (331) g; *P* = 0.150) between Indigenous female and male neonates. There was no significant difference in TRV between male and female non-Indigenous neonates (22.0 (4.7) *vs* 20.8 (5.5) mL; *P* = 0.404) (Fig. 4). Multiple linear regression model indicated that TRV was positively correlated to weight at term corrected but not gestation or birth weight (Table 1).

Fig. 2 No difference in body weight at term corrected. The vertical axis is body weight (g).

Fig. 1 At term corrected, Indigenous neonates had smaller renal volumes.

Fig. 3 No difference in eGFR at term corrected (ml/min⁻¹ per 1.73 m²).
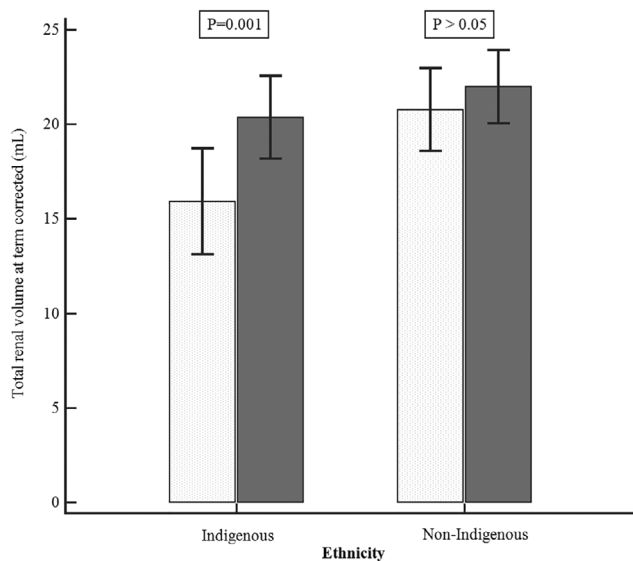
Fig. 4 Female Indigenous neonates have smaller renal volume (Mean (SD)) compared to Indigenous male neonates at term corrected, but this difference is not seen in the Non-Indigenous population. (□) Female, (▩) male.

Table 1 Multiple linear regression analysis for total renal volume at term corrected

Independent variables	Coefficient	P
Constant	−2.066	
Birth weight (g)	0.0012	0.707
Gestation (weeks)	0.360	0.344
Weight at term corrected	0.005	**0.004**

DISCUSSION

At term corrected gestational age, Indigenous preterm neonates had significantly smaller renal volumes, and therefore they had a lower N_{Glom} on average than non-Indigenous preterm neonates. Despite fewer N_{Glom}, Indigenous neonates had similar eGFR to non-Indigenous neonates. Total eGFR is the product of the single-nephron GFR $\times$ N_{Glom}.[22] Hence, Indigenous preterm neonates achieved similar eGFR through single nephron hyperfiltration, a concept first proposed by Brenner.[23]

Our data also found that female Indigenous neonates had smaller renal volumes compared with Indigenous male preterm neonates, but there were no differences in birth weight, gestational age, renal function and weight at term corrected, between these two groups. Other investigators have previously reported a difference in renal volume between male and female Indigenous children. Spencer et al.[24] evaluated a cohort of 174 children aged between 5 and 18 years in an Aboriginal community with a high prevalence of renal diseases in Northern Territory, Australia. The investigators found that male Indigenous children had larger renal volume adjusted to body surface area when compared to female Indigenous children. Hoy et al.[25] demonstrated that Indigenous adults had 30% fewer glomeruli

(202 000 fewer glomeruli per kidney, or an estimated 404 000 fewer nephrons/individual, ($P = 0.036$),[25,26] with a mean glomerular volume that is 27% larger than that of the non-Indigenous group ($P = 0.016$). Their data also showed that female Indigenous persons had 5% fewer nephrons when compared with male Indigenous persons.[25]

While Indigenous status is an important determinant of CKD in the Australian population, gender is an important determinant of the need for renal dialysis.[27] In remote Australia, the incidence of Indigenous women needing dialysis is higher compared with Indigenous men.[28] A retrospective cohort analysis, Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry from 2001 to 2013 (n = 21 832) reported that the incidence of dialysis in Indigenous women was higher than in Indigenous men (513 per million population and 406 per million population, respectively).[27] The reverse was reported among non-Indigenous people. Death associated with renal diseases between 1998 and 2012 was 3.9 times higher in Indigenous females and 2.6 times higher for Indigenous males when to compared with non-Indigenous Australians.[28] The causes of these disparities need to be fully understood.

Reduced nephron endowment is likely to be an important risk factor for CKD among Indigenous people. Compounding factors during childhood and adolescence would further set an Indigenous infant on a trajectory to develop CKD and end-stage renal disease at a younger age compared with their non-Indigenous Australian contemporaries. Hoy et al.[29] investigated the association between episodes of acute post streptococcal glomerulonephritis (PSGN) and the risk for CKD in later life in a cohort of 1519 residents of a remote Australian Indigenous community, with high rates of cardiovascular and renal disease. Approximately 13% (200/1519) of the residents had at least one episode of PSGN and, numerous episodes during childhood in 27 others. Elevated ACR level was noted with increasing age. Group A streptococcal skin infections, often related to scabies were identified in all PSGN episodes. For men and women aged 10–39 years, approximately one in five had PSGN. PSGN (5 years or more before assessment) showed a significant correlation with ACR. A history of PSGN among women aged 30–39 had a significant correlation with reduced GFR. In both genders with a history of PSGN, the adjusted odds ratios for an ACR to be above 34 g/mol (overt albuminuria) was 4.6 (men) and 3.1 (women). The investigators in this study concluded that PSGN contributes significantly to the increased risk of CKD in that community.[29]

There are a few limitations to our data. Some of the neonates were transferred back to hospitals nearer to their homes before discharge from the hospital. Hence, less than a third of the eligible patients were recruited into this study. The total number of admissions during this study period was 375 neonates, out of which 139 were Indigenous (139/375 = 37%). In the final data set, data from 105 neonates were analyzed, out of which 38 were indigenous (38/105 = 36%). Hence,

while we were unable to eliminate bias, we believe that the effect of selection bias in our data is minimal. We considered all neonates from the Aboriginal and Torres Strait Islander community as a single group and did not make any attempt to divide this group further. We used TRV as a proxy indicator of nephron number, and the use of CysC as a marker of renal function in neonates is currently limited. We are currently following up these neonates until the age of 24 months.

In conclusion, Indigenous neonates are more premature and smaller compared to non-Indigenous neonates. At term corrected age, premature Indigenous neonates have smaller renal volumes (hence nephron number) but similar eGFR. Thus, they achieve similar eGFR through hyperfiltration, which increases their risk of developing CKD over their lifetime. Female Indigenous neonates have smaller renal volumes compared to Indigenous male neonates. We believe this is an important reason for the difference in morbidity and mortality from the renal disease between male and female Indigenous adults.

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DISCLOSURE

We have no conflict of interest to report

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