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# Soluble (pro)renin receptor (s(P)RR) levels in women carrying Aboriginal and/or Torres Strait Islander babies; the Gomeroi Gaaynggal study

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ABSTRACT

*Objective:* To determine the levels of soluble (pro)renin receptor (s(P)RR) in women carrying Aboriginal and/or Torres Strait Islander (First Nations) babies and investigate whether s(P)RR levels change in women who have complicated pregnancies.

*Study Design:* Cross-sectional analysis of data (2010–2018). Data/samples were from the Gomeroi Gaaynggal Study, a longitudinal cohort study based on Gomeroi/Kamilaroi lands (Tamworth), NSW, Australia. Third trimester samples (blood/urine) were collected from pregnant women carrying a First Nations baby (N = 188).

*Methods/Main outcome measures:* Plasma s(P)RR and markers of kidney function (plasma: creatinine, urea and cystatin C; urinary: creatinine, protein, albumin, angiotensinogen, nephrin and Na/K) were measured by enzyme-linked immunosorbent assay or standardised pathology procedures as needed.

*Results:* Soluble (P)RR was detected in plasma of women in the cohort (median: 19.86 ng/mL; IQR: 12.52–26.8). Soluble (P)RR levels correlated positively with maternal plasma creatinine (P = 0.0001) and gestational age in the third trimester (P = 0.002). Levels of s(P)RR tended to positively correlate with urinary protein/creatinine (P = 0.04) and nephrin/creatinine (P = 0.03). Soluble (P)RR levels tended to be higher in women who birthed prematurely (P = 0.06). Soluble (P)RR levels did not change with other pregnancy complications or outcomes (preeclampsia, GDM or small or large for gestational age birth).

*Conclusions:* Soluble (P)RR is present in the plasma of pregnant women carrying First Nations babies and is correlated with known urinary biomarkers of renal function. Increased maternal s(P)RR levels may be associated with increased risk of preterm birth.

## 1. Introduction

The (pro)renin receptor ((P)RR) is a multifunctioning protein and component of the renin angiotensin system (RAS) [1], which is a critical system for blood pressure and electrolyte homeostasis and for maintaining a healthy pregnancy. The extracellular domain of the (P)RR can be proteolytically cleaved [2–5] to form a novel 28 kDa protein, the soluble (pro)renin receptor (s(P)RR). The s(P)RR can mimic RAS signalling by either binding renin and thereby activating the RAS cascade [2], or by directly binding and activating the angiotensin II type 1 receptor [6]. Soluble (P)RR has been detected in both plasma [2] and urine [7], and has been shown to be involved in blood pressure regulation [6,8,9]. Soluble (P)RR is of interest as both a potential novel biomarker for RAS dysfunction and as a protein of interest in the pathophysiology of hypertensive pathologies. Very little is known however about the functions of s(P)RR in human pregnancy.

Levels of s(P)RR in the maternal plasma of pregnant women increase with gestational age [10]. This is however contested by Mikami *et al.*, who showed that s(P)RR levels only correlate with gestational age in women who have pregnancy complications [11]. Soluble (P)RR levels in

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the maternal plasma of women with preeclampsia and gestational diabetes mellitus (GDM) are also elevated, implicating s(P)RR in the pathology of preeclampsia and GDM [10,12–14], although this has been disputed by Sugulle *et al.,* who did not find increased s(P)RR levels in preeclamptic pregnancies [13]. These differences may be due to the ethnic disparities between studies or differences in the diagnostic criteria or severity of preeclampsia. In both non-pregnant mice and pregnant rats, treatment of animals with s(P)RR induces endothelial dysfunction, alongside hypertension [6,15], suggesting that s(P)RR may also have a causal role in the pathophysiology of preeclampsia.

Soluble (P)RR levels are elevated in the plasma of participants who have chronic kidney disease, heart failure [1], preeclampsia [12] and GDM [13,14]. All these conditions are more prevalent in Australian Aboriginal and Torres Strait Islander people (hereafter respectfully referred to as First Nations) [16,17]. However, circulating levels of s(P)RR have never been measured in First Nations Australians or during the pregnancies of First Nation women. Furthermore, circulating maternal s(P)RR levels have never been measured in any women, regardless of First Nation status, who have delivered preterm, and/or have small (SGA) or large for gestational age (LGA) infants.

In this study, we aimed to measure the levels of s(P)RR in pregnant women carrying First Nations babies and determine if s(P)RR levels are altered in women with pregnancy/birth pathologies (including preeclampsia, GDM, preterm birth, SGA and LGA). Considering that s(P)RR has also been associated with renal dysfunction [18], which disproportionately affects First Nations people [19], we then assessed whether s(P)RR was associated with markers of renal function and investigated changes in women who had pregnancy complications. We hypothesised that s(P)RR levels would be altered in complicated pregnancies and that s(P)RR levels would be associated with markers of renal function.

## 2. Methods

### 2.1. Ethics and community consultation

This study was approved by the Hunter New England Human Research Ethics Committee (08/05/21/4.01); the New South Wales Human Research Ethics Committee (HREC/08/HNE/129); the Aboriginal Health and Medical Research Council Human Research Ethics committee (654/08); and the University of Newcastle Human Research Ethics Committee (H-2009–0177). The Gomeroi Gaaynggal Study was developed in consultation with the community [20]. The Gomeroi Gaaynggal Advisory Committee approved the publication of this article.

### 2.2. Study design

The Gomeroi Gaaynggal Study is a longitudinal cohort study that follows mothers carrying First Nation infants, from pregnancy through to early childhood. Recruitment for this study was conducted by First Nation research assistants who recruited participants at antenatal clinics primarily located in Tamworth, New South Wales, Australia. Written and informed consent was obtained from all participants. During pregnancy, participants engaged with First Nation research assistants once per trimester when biological samples, surveys and physiological measurements were taken. Where possible, these were all taken by First Nation research assistants. Further details describing the study have been published elsewhere [20].

The data used in this study are a subset of data collected as part of the larger Gomeroi Gaaynggal Study and includes women who birthed between the years of 2010–2018. Women who had a multiple pregnancy (i.e. twins etc.) were excluded from this data set. Only those who had a s(P)RR measurement in the third trimester were included in the final analysis. Additionally, if women participated in the study more than once (i.e. had more than one pregnancy in the study) only their first complicated pregnancy was included, if there were no pregnancy complications then their first pregnancy was included (N = 188).

### 2.3. Demographics, maternal and fetal characteristics data collection

Data concerning participants' demographics and maternal and fetal characteristics were collected from the participants medical records. Where details were missing from the participants' medical records, information was collected from participant surveys conducted throughout the study. Information on First Nation status and maternal age were collected directly from participants upon recruitment.

Blood pressure was measured with patients seated using a Riester rechampion® blood pressure machine and cuff as previously described in Ashman et. al. [20].

Birthweight centiles were calculated using the Gestation Related Optimal Weight (GROW) Global Bulk Centile Calculator (Version 8.0.6.1). The Australasian mean maternal weight and height (69.1 kg and 166 cm, respectively) and a parity of one was used when there were missing data (as dictated by the GROW centile calculator).

### 2.4. Blood sample collection and preparation

Blood samples were collected from non-fasting participants in the third trimester of pregnancy via venepuncture. Samples were then transiently stored in EDTA vacutainers and handled at room temperature until centrifugation (40X RPM for 10 min). After centrifugation plasma was collected, snap frozen and stored at −80 °C for further analyses.

### 2.5. Urine sample collection and preparation

Urine was self-collected by participants, temporarily stored on ice, then aliquoted, snap frozen and stored at −80 °C for further analyses.

### 2.6. Enzyme linked immunosorbent assay (ELISA)

Maternal plasma s(P)RR levels (catalogue number 27782; IBL, USA) and maternal urinary Nephrin and AGT levels (catalogue numbers DY4269-05 and DY3156, respectively; both R & D Systems, USA) were measured via ELISA according to the manufacturer's instructions. Optical densities were measured using a SPECTROstar$^{nano}$ microplate reader. The inter- and intra-assay coefficient of variation was < 15 % for all assays/samples.

### 2.7. Measurement of urea, electrolytes, and creatinine

Urinary and plasma creatinine and urinary protein and albumin were measured using Siemens Flex reagent cartridges and read using a Siemens Dimension Vista 1500 chemistry analyser at NSW Health Pathology Tamworth (NSW, Australia). Urinary protein and albumin were normalised to urinary creatinine. Plasma cystatin C and urea and urinary sodium and potassium were measured using standardized pathology protocols.

### 2.8. Data grouping

Participants' data were grouped according to pregnancy and birth outcomes. A control group was created of women who did not give birth to small for gestational age (SGA; <10th centile), large for gestational age (LGA; >90th centile) or preterm (<37 week) infants and who did not have any form of diabetes (type 1, type 2, or gestational), or hypertension (essential, gestational, or preeclampsia/eclampsia; N = 85). Women who gave birth to preterm infants were grouped separately regardless of any other pregnancy complication or birth outcome (N = 14). Women who gave birth to infants who were SGA or LGA and who did not give birth to preterm infants or have hypertension or diabetes were grouped separately (N = 24 and 16, respectively). Women who had any form of diabetes (N = 22), any form of hypertension (N = 13), or who had both diabetes and hypertension (N = 5) were grouped

**Table 1**
Maternal and fetal characteristics.

| Variable | | Data available (N (%)) | Median (IQR) | N (%) |
|---|---|---|---|---|
| **Maternal** | | | | |
| First Nations status | | 188 (100) | N/A | |
| | First Nations mother | | | 100 (53.2) |
| | First Nations father | | | 33 (17.6) |
| | Both First Nations | | | 55 (29.3) |
| Pre-pregnancy BMI (kg/m$^2$) | | 154 (81.9) | 28.0 (22.8–34.6) | |
| | Underweight (<18.5) | | *17.3 (16.9–17.9)* | 9 (5.8) |
| | Healthy weight (18.5–24.9) | | *22.4 (20.2–23.3)* | 48 (31.2) |
| | Overweight (25–29.9) | | *27.6 (26.2–28.6)* | 32 (20.8) |
| | Obese (>30) | | *36.2 (32.6–40.7)* | 65 (42.2) |
| Smoking | | 132 (70.2) | N/A | |
| | Non-smoker | | | 94 (71.2) |
| | Smoker | | | 38 (28.8) |
| Age at blood draw (years) | | 188 (100) | 24.4 (20.7–29.5) | |
| | <20 | | | 34 (18.1) |
| | 20–34.9 | | | 135 (71.8) |
| | >35 | | | 19 (10.1) |
| Gravidity | | 121 (64.4) | 2 (1–5) | N/A |
| Parity | | 121 (64.4) | 2 (1–3) | N/A |
| Hypertension | | 178 (94.7) | N/A | |
| | None | | | 160 (89.9) |
| | Chronic | | | 1 (0.6) |
| | Pregnancy induced | | | 7 (3.9) |
| | Preeclampsia | | | 10 (5.6) |
| Diabetes | | 174 (92.6) | N/A | |
| | None | | | 147 (84.5) |
| | Type 1 | | | 1 (0.6) |
| | Type 2 | | | 3 (1.7) |
| | GDM | | | 23 (13.2) |
| Gestational age at blood draw (weeks) | | 188 (100) | 35.4 (32.7–37.1) | N/A |
| Gestational age at delivery (weeks) | | 173 (92.0) | 39.1 (38.2–40.2) | |
| | Preterm (<37) | | *35.9 (35.2–36.5)* | 14 (8.1) |
| | Term (37–41.9) | | *39.4 (38.9–40.2)* | 159 (91.9) |
| | Post term (≥42) | | | 0 (0) |
| **Fetal** | | | | |
| Birthweight (g) | | 173 (92.0) | 3390 (2950–3685) | N/A |
| GROW birthweight centile | | 173 (92.0) | 44.6 (13.9–71.2) | |
| | SGA (<10th centile) | | *4.2 (1.9–6.8)* | 29 (16.8) |
| | AGA (10-90th centile) | | *45.2 (27.1–67)* | 122 (70.5) |
| | LGA (>90th centile) | | *96.9 (92.3–99.3)* | 22 (12.7) |
| Sex | | 173 (92.0) | N/A | |
| | Male | | | 96 (55.5) |
| | Female | | | 77 (44.5) |

IQR = interquartile range; BMI = body mass index; GDM = gestational diabetes mellitus;
SGA = small for gestational age; AGA = appropriate for gestational age;
LGA = large for gestational age.

separately, these groups included women who also gave birth to SGA/LGA or preterm infants.

### 2.9. Data analysis

Statistical analyses were performed using statistics and data science (Stata) basic edition (BE) Version 17.0. Non-parametric statistics were used for all data and $P < 0.05$ was classified as significant. Spearman's rank correlation tests with Bonferroni correction applied were used to determine significant correlations between s(P)RR levels and physiological measurements. The bonferroni corrected P value was calculated at $P < 0.003$ for all correlations. Mann-Whitney tests were used to determine significant differences in maternal and fetal characteristics and s(P)RR levels between the preterm and control groups. Kruskal-Wallis statistical tests with Dunn's pairwise comparisons were used to determine significant differences between both SGA, LGA and control groups and diabetic, hypertensive, diabetic/hypertensive, and control groups, in maternal and fetal characteristics and s(P)RR levels.

## 3. Results

### 3.1. Maternal and fetal characteristics

The maternal characteristics of this cohort can be seen in Table 1. Briefly, most mothers identified as being First Nations (82.5 %), however, 17.6 % did not identify as First Nations or their status was unknown but the father of their baby was identified as First Nations. The median BMI for the cohort was 28 kg/m$^2$. Most women in the study were non-smokers (71.2 %), aged between 20–34.9 years (71.8 %) and did not have hypertension or diabetes (89.9 % and 84.5 %, respectively). The prevalence of preeclampsia, pregnancy-induced hypertension and chronic hypertension in the cohort was 5.5 %, 3.9 % and 0.6 %, respectively. The prevalence of gestational diabetes mellitus, Type 2 and Type 1 diabetes in the cohort was 13.2 %, 1.7 % and 0.6 %, respectively. The median gestational age when s(P)RR was measured was 35.4 weeks. Most women (91.9 %) delivered at term.

Fetal characteristics are also described in Table 1. The median birthweight of infants in the study was 3390 g. SGA accounted for 16.8 % of infants, 12.7 % of infants were LGA.

### 3.2. Pregnancy and birth outcomes and their relation to maternal and fetal characteristics

Differences in maternal characteristics between groups are listed in Table 2. Briefly, maternal BMI and age were higher in the diabetic ($P = 0.00$ and $P = 0.003$, respectively) and combined diabetic/hypertensive ($P = 0.02$ and $P = 0.03$, respectively) groups compared with control. Systolic and diastolic blood pressures were increased in the hypertensive group compared with control ($P = 0.0002$ and $P = 0.002$, respectively). Gestational age at delivery was significantly decreased in the preterm ($P = 0.00$), diabetic ($P = 0.00$), hypertensive ($P = 0.002$), and combined diabetic/hypertensive groups ($P = 0.005$) compared with control.

Differences in fetal characteristics between all groups are listed in Table 2. Briefly, birthweight at delivery was significantly less in the preterm ($P = 0.00$), SGA ($P = 0.00$) and hypertensive ($P = 0.0006$) groups and significantly higher in the LGA group ($P = 0.00$), compared with control. Birthweight centiles were significantly lower in the SGA and hypertensive groups ($P = 0.00$ and $P = 0.02$, respectively) and higher in the LGA group ($P = 0.00$), compared with the control.

**Table 2**
Comparisons between pregnancy and birth outcomes and maternal and fetal characteristics.

| | Control N = 85 | | Preterm N = 14 | | SGA N = 24 | | LGA N = 16 | | Diabetes N = 22 | | HTN N = 13 | | Diabetes and HTN N = 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Maternal** | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) |
| Pre-pregnancy BMI ($kg/m^2$) | 78 | 26.89 (22.5–32.51) | 11 | 27.77 (20.96–37.65) | 17 | 30.78 (25.33–37.20) | 15 | 27.18 (22.5–34.89) | 21 | ***33.43 (30.85–43.82)***** | 11 | 22.86 (20.20–27.97) | 5 | ***38.47 (28.04–45.54)**** |
| Age at blood draw (years) | 85 | 23.9 (20.58–28.16) | 14 | 22.2 (20.76–31.1) | 24 | 24.62 (22.27–29.44) | 16 | 23.49 (20.83–26.67) | 22 | ***32.23 (23.68–34.33)**** | 13 | 22.21 (19.87–27.46) | 5 | ***31.15 (29.36–32.44)**** |
| Systolic blood pressure (mmHg) | 72 | 114 (107–122) | 13 | 123 (115–130) | 16 | 116 (110–120.5) | 14 | 112.5 (110–120) | 18 | 116 (112–124) | 12 | ***127 (121.5–134)**** | 4 | 118 (113–123.5) |
| Diastolic blood pressure (mmHg) | 72 | 73.5 (65–78.5) | 13 | 71 (69–80) | 16 | 70.5 (62–77.5) | 14 | 70 (64–74) | 18 | 74 (68–80) | 12 | ***85.5 (74–91)**** | 4 | 70.5 (70–73) |
| Parity | 58 | 1 (1–2) | 8 | 1 (0–3.5) | 12 | 2 (1–3.5) | 11 | 1 (0–2) | 17 | 2 (1–3) | 8 | 1 (0–1.5) | 4 | 3.5 (2–5) |
| Gestational age at delivery (weeks) | 85 | 39.5 (38.86–40.2) | 14 | ***35.96 (35.2–36.5)***** | 24 | 39.25 (38.1–40.54) | 16 | 39.5 (38.5–40.25) | 22 | ***38.4 (38.1–39.1)***** | 13 | ***38.3 (37.6–39)**** | 5 | ***37.29 (35.6–38)**** |
| **Fetal** | | | | | | | | | | | | | | |
| Birthweight (g) | 85 | 3510 (3260–3685) | 14 | ***2720 (2400–2954)***** | 24 | ***2850 (2537.5–3065)***** | 16 | ***4227.5 (3927.5–4500)***** | 22 | 3465 (3155–3670) | 13 | ***3130 (2735–3315)**** | 5 | 2960 (2870–4120) |
| GROW birthweight centile | 85 | 45.6 (28–67) | 14 | 50.05 (13–78.3) | 24 | ***4.7 (1.4–6.8)***** | 16 | ***97.25 (93.05–99.05)***** | 22 | 61.85 (34.4–74.9) | 13 | ***25.4 (10.3–45.1)**** | 5 | 68.5 (60.2–78.3) |

* indicates a statistically significant difference between the group variable and the corresponding control group variable where; *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.0001$. SGA = small for gestational age; LGA = large for gestational age; HTN = hypertension; IQR = interquartile range; BMI = body mass index;

**Table 3**
Correlations between soluble prorenin receptor levels and physiological measurements.

| Variable | r | P | N (%) |
|---|---|---|---|
| *s(P)RR levels (ng/mL)* | | | |
| Blood Pressure (mmHg) | | | |
| Systolic | 0.12 | 0.13 | 149 (79.3) |
| Diastolic | 0.05 | 0.52 | 149 (79.3) |
| Maternal | | | |
| Age (at blood draw; years) | 0.03 | 0.73 | 188 (100) |
| BMI (prepregnancy; kg/m2) | −0.03 | 0.68 | 154 (81.9) |
| Plasma creatinine (umol/L) | 0.28 | ***0.0001**** | 187 (99.5) |
| Plasma urea (mmol/L) | 0.09 | 0.22 | 186 (98.9) |
| Plasma Cystatin C (mg/L) | 0.06 | 0.45 | 169 (89.9) |
| Gestational age at blood draw (weeks) | 0.23 | ***0.002**** | 188 (100) |
| Maternal urine | | | |
| Protein/creatinine (mg/mmol) | 0.17 | 0.04 | 157 (83.5) |
| Albumin/creatinine (mg/mmol) | −0.05 | 0.5 | 159 (84.6) |
| Na/k (mmol/L) | −0.11 | 0.19 | 159 (84.6) |
| AGT/creatinine (ng/mL/mmol) | −0.01 | 0.16 | 159 (84.6) |
| Nephrin/creatinine (ng/mL/mmol) | 0.22 | 0.03 | 100 (53.2) |
| Infant | | | |
| Birthweight (g) | 0.05 | 0.55 | 173 (92.0) |
| GROW Birthweight Centile | 0.09 | 0.24 | 173 (92.0) |

BMI = body mass index; AGT = angiotensinogen.
Bonferroni adjusted significance value of $P < 0.003$ was used to account for the increased possibility of a type-I error.

### 3.3. Correlations between s(P)RR levels and physiological measurements of the cohort

The median maternal plasma s(P)RR level was 19.86 ng/mL (IQR = 12.52–26.8). Correlations between s(P)RR levels and physiological measurements are seen in Table 3. The Bonferroni adjusted significance value ($P < 0.003$) was calculated to account for the increased possibility of a type-1 error. Briefly, s(P)RR levels positively correlated with maternal plasma creatinine ($P = 0.0001$) and gestational age at blood draw ($P = 0.002$). Soluble (P)RR levels tended to positively correlate with urinary protein/creatinine ($P = 0.04$) and nephrin/creatinine levels ($P = 0.03$), however these did not reach statistical significance after bonferroni correction. There were no other significant correlations.

### 3.4. Soluble (P)RR levels in pregnancy complications

Soluble (P)RR levels did not change between women who gave birth to SGA or LGA babies or had pregnancies complicated by diabetes, hypertension or diabetes/hypertension and the control group (Fig. 1*A and B*).

Soluble (P)RR levels tended to be increased in women who gave birth preterm compared with those who birthed at term ($P = 0.06$; Fig. 1*C*). Soluble (P)RR levels tended to be positively correlated with gestational age in the term group ($P = 0.04$) but did not reach Bonferroni adjusted statistical significance (Fig. 1*D*).

### 3.5. Pregnancy and birth outcomes and their relation to markers of kidney dysfunction

Maternal urinary protein/creatinine was significantly increased in the preterm ($P = 0.008$), hypertension ($P = 0.005$) and diabetes/hypertension ($P = 0.03$) groups compared with the control group (Table 4). Maternal urinary albumin/creatinine was significantly increased in the diabetic ($P = 0.03$), hypertensive ($P = 0.03$) and diabetic/hypertensive ($P = 0.004$) groups compared with the control group (Table 4). There were no other significant differences.

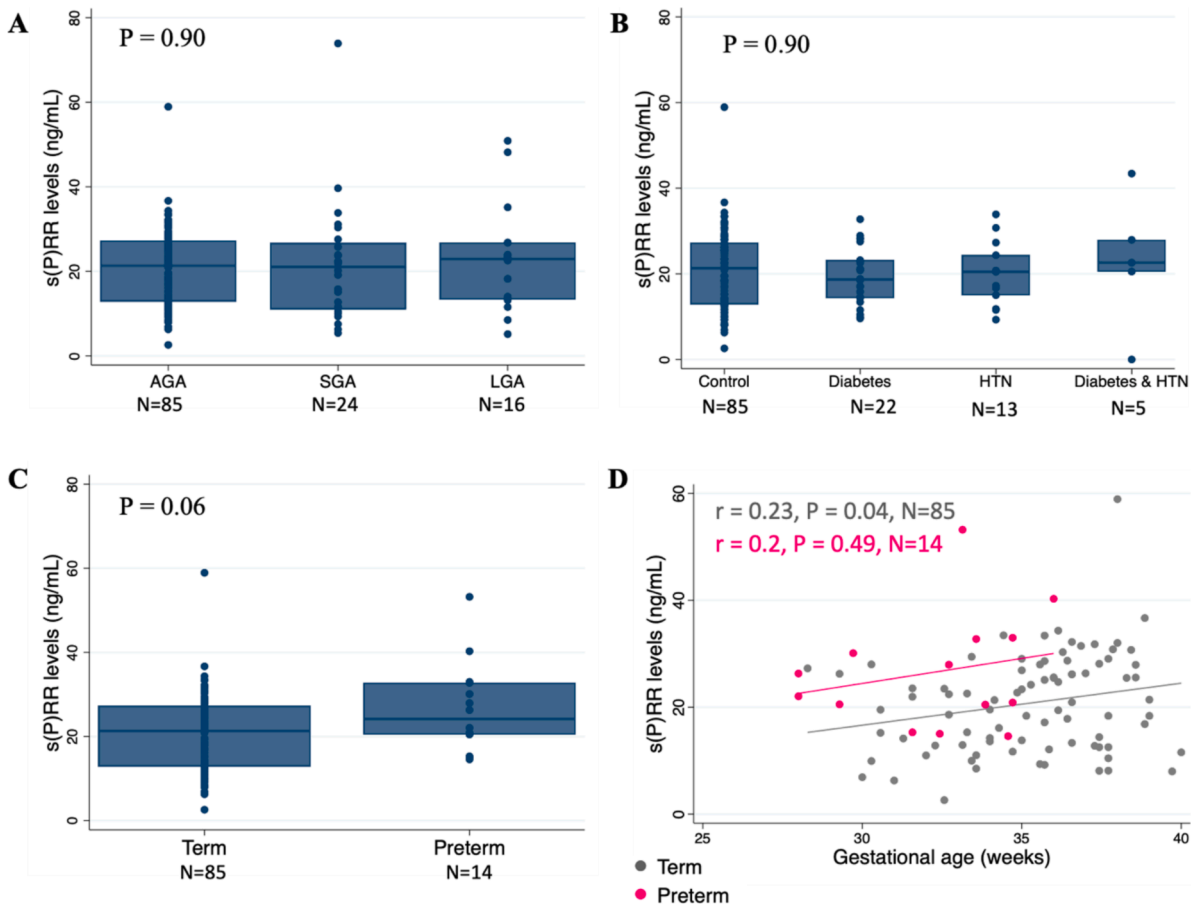


**Fig. 1.** *Levels of soluble (pro)renin receptor (s(P)RR) in particpants with pregnancy complications.* (**A**) Soluble (P)RR levels were not affected by infants being small or large for gestational age (SGA/LGA) compared with appropriately grown (AGA) controls. Additionally, (**B**) hypertension (HTN), diabetes or the combination of hypertension and diabetes in pregnancy did not affect s(P)RR levels when compared with controls. (**C**) s(P)RR levels tended to be increased in women who subsequently gave birth preterm when compared with women who birthed at term. (**D**) Soluble (P)RR levels tended to be positively correlated with gestational age in the third trimester in the term control group (P = 0.04), but not the preterm group. Bonferroni adjusted significance value of P < 0.003 was used to determine a significant correlation between s(P)RR levels and gestational age. Data are presented as median and interquartile range.

## 4. Discussion

We are the first to establish plasma s(P)RR levels in pregnant women carrying First Nations babies. We have shown that plasma levels of s(P)RR in this cohort correlate with markers of kidney function and with gestational age in the third trimester. Third trimester s(P)RR levels tended to be increased in women who gave birth preterm. Soluble (P)RR levels were not changed in women with any other pregnancy complications or birth outcomes (preeclampsia, GDM, SGA or LGA).

Very little is known about how s(P)RR levels relate to physiological measures in pregnancy, particularly in First Nations women. We have shown that s(P)RR levels do not correlate with maternal systolic or diastolic blood pressure in the third trimester (Table 3), supporting the current literature [10,12]. Interestingly, Watanabe *et al.,* found elevated s(P)RR levels in early pregnancy predicted elevations in blood pressure in later pregnancy [10]; this needs to be further investigated in our cohort. Furthermore, we showed that s(P)RR levels positively correlated with gestational age, again congruent with findings by Watanabe *et al.,* [10].

Elevated s(P)RR levels may be associated with kidney dysfunction in this cohort. We have shown that s(P)RR levels positively correlated with serum creatinine levels (Table 3) and tended to associate with urinary protein/creatinine and nephrin/creatinine levels in the third trimester. Our data aligns with a previous study by Morimoto *et al.,* of non-pregnant patients with essential hypertension [21]. It should be noted however that serum creatinine is rarely used as a sole marker of kidney dysfunction and is regularly looked at in conjunction with plasma cystatin C [22], which did not correlate correspondingly with s(P)RR levels. Importantly, the s(P)RR has previously been shown to activate the RAS and play a role in sodium reabsorption in the kidney [18]. In this way, the s(P)RR may have both direct and indirect roles in the development of kidney dysfunction and hence could account for the positive correlation between s(P)RR and kidney function markers in this study [18]. Additionally, the concurrent association between both urinary nephrin and protein/creatinine and plasma s(P)RR levels, likely reflects an association between increased s(P)RR levels and podocyte injury [23].

We found no changes in plasma s(P)RR levels in women who birthed SGA/LGA infants compared with AGA controls (Fig. 1), nor were these birth outcomes associated with changes in kidney function markers (Table 4). This study is, to our knowledge, the first to measure maternal levels of s(P)RR in women who birthed SGA/LGA infants. Watanabe *et al.,* has shown that high cord blood levels of s(P)RR are associated with reduced likelihood of SGA birth [24]. This is likely due to increased fetal s(P)RR as s(P)RR is too large (28 kDa) to cross the placental barrier.

Plasma s(P)RR levels were not different in pregnant women with hypertension, diabetes or both compared with normotensive controls (Fig. 1). Our findings support those by Sugulle *et al.,* [13], however Sugulle *et.al.*, also observed an increase in s(P)RR levels in GDM which we did not. Our findings also contradict findings by others showing that s(P)RR levels are elevated in women with preeclampsia [10,12] or GDM [13,14]. Normotensive third trimester women in this cohort tend to have lower levels of plasma s(P)RR (21.3 ng/ml (12.9–27.3) ng/ml;

**Table 4**
Comparisons between pregnancy and birth outcomes and markers of kidney dysfunction.

| | Control N = 85 | | Preterm N = 14 | | SGA N = 24 | | LGA N = 16 | | Diabetes N = 22 | | HTN N = 13 | | Diabetes and HTN N = 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Maternal Urine** | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) |
| Protein/ creatinine (mg/mmol) | 73 | 0.01 (0.01–0.02) | 13 | ***0.02*** ***(0.01–0.03)*** ** | 19 | 0.01 (0.01–0.02) | 14 | 0.02 (0.01–0.02) | 18 | 0.02 (0.01–0.02) | 11 | ***0.02*** ***(0.01–0.3)*** *** | 4 | ***0.04*** ***(0.02–0.05)**** |
| Albumin/ creatinine (mg/mmol) | 75 | 1.04 (0.75–1.79) | 13 | 1.61 (1.01–2.38) | 19 | 1.33 (0.71–2.85) | 14 | 1.13 (0.79–2.44) | 18 | ***1.51*** ***(0.81–3.12)*** * | 11 | ***2.08*** ***(0.98–3.97)*** * | 4 | ***8.97*** ***(3.3–19.95)*** *** |
| Na/K (mmol/L) | 74 | 1.78 (1.33–2.9) | 13 | 1.55 (1.31–2.06) | 19 | 1.62 (1.06–2.38) | 14 | 1.62 (0.62–2.15) | 19 | 1.32 (1.01–2.2) | 11 | 2.28 (1.09–2.5) | 4 | 1.12 (0.92–1.72) |
| AGT/ creatinine (ng/mL/ mmol) | 75 | 0.36 (0–3.55) | 13 | 0.05 (0–0.95) | 19 | 0.01 (0–1.59) | 14 | 0.26 (0–3.01) | 18 | 3.22 (0.42–10.66) | 11 | 1.33 (0–4.13) | 4 | 18.85 (4.35–30.25) |
| Nephrin/ creatinine (ng/mL/ mmol) | 46 | 0.02 (0.01–0.04) | 8 | 0.02 (0.01–0.08) | 10 | 0.04 (0.02–0.11) | 9 | 0.03 (0–0.05) | 15 | 0.02 (0–0.03) | 8 | 0.03 (0–0.04) | 3 | 0.01 (0–0.01) |
| **Maternal Plasma** | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) | N | median (IQR) |
| Plasma creatinine (umol/L) | 85 | 51 (46–55) | 14 | 51 (49–57) | 24 | 51 (46–54) | 15 | 48 (45–52) | 22 | 51 (49–54) | 13 | 53 (44–56) | 5 | 51 (51–58) |
| Plasma urea (mmol/L) | 85 | 2.4 (2–3.1) | 14 | 2.35 (2–3.1) | 24 | 2.65 (2.1–2.95) | 15 | 2.4 (1.9–3.1) | 22 | 2.4 (2.1–3) | 13 | 2.3 (1.9–3.3) | 5 | 3.5 (3.1–3.6) |

* indicates a statistically significant difference between the group variable and the corresponding control group variable where; *=P < 0.05, **= P < 0.01, ***=P < 0.0001. SGA = small for gestational age; LGA = large for gestational age; HTN = hypertension; IQR = interquartile range; Na = Sodium; K = Potassium; AGT = Angiotensinogen.

median (IQR)), than those reported in other cohorts (24.9 ± 3.7 and 39.2 ± 8.9 ng/ml [10,12]). Correspondingly, plasma s(P)RR levels in hypertensive women in their third trimester are also markedly lower in this study (20.5 ng/ml (15.0–24.4) ng/ml; median (IQR)) than those reported by others in the third trimester of pregnancies complicated by preeclampsia (32 ± 10.6 ng/mL) [12]. In terms of GDM, Watanabe *et. al.*, have reported that first trimester women later diagnosed with GDM had mean s(P)RR levels of 35.5 ± 22.7 ng/ml [14]. It is, however, difficult to compare these with the levels found in our study since we measured third trimester s(P)RR levels in women with any form of diabetes (18.7 (14.4–23.2) ng/mL; median (IQR)) and we (and others) have shown that s(P)RR levels change with gestational age [10]. Differences in diagnostic criteria, ethnicity, maternal/fetal characteristics, or severity of hypertension and/or diabetes between studies could account for discrepancies between our study and the wider literature. Importantly, these data highlight that s(P)RR may not be a suitable biomarker for preeclampsia or GDM in pregnant women carrying First Nation infants.

Protein/creatinine levels were significantly higher in women who had pregnancies complicated by hypertension and both hypertension and diabetes (Table 4). Additionally, albumin/creatinine levels were significantly increased across all diabetic and/or hypertensive groups (Table 4). Elevated protein/creatinine and albumin/creatinine levels are commonly observed in pregnancies complicated by preeclampsia and GDM [25,26]. Increased albumin/creatinine levels in women with pregnancies complicated by GDM have been hypothesised to be predictive of cardiovascular disease (CVD) in later life [25]. Therefore, it would be interesting to follow-up women in this cohort to determine if their risk of CVD is higher after having GDM, as has been seen after preeclampsia [27].

Additionally, women diagnosed with both hypertension and diabetes in pregnancy also had higher AGT/creatinine levels than normotensive women (although this did not reach statistical significance, probably due to the low N = 5). AGT/creatinine is a novel kidney function marker reflecting intrarenal RAS activity and is increased in chronic kidney disease [28]. These results corroborate our previous findings that urinary AGT/creatinine levels do not change in women with a complicated pregnancy compared with normotensive women [29]. However, this previous study did not differentiate between pregnancy complications and did not include any First Nations women with both hypertension and diabetes. This data indicates that pregnant women with both hypertension and diabetes in this cohort had evidence of more severe kidney dysfunction.

We have shown that plasma s(P)RR levels tend to be increased in the third trimester of women who later birthed preterm compared with term (Fig. 1C; median s(P)RR levels in the preterm group were: 24.2 ng/mL (20.5–32.8); (median (IQR)). Interestingly, urinary protein/creatinine levels in the preterm group were also increased compared with the term group (Table 4). Proteinuria in pregnancy has been associated with preterm labour [30], particularly in preeclamptic women [31]. Additionally, as discussed above, the s(P)RR may play an active role in the development of kidney dysfunction [18]. Hence it is possible that increased s(P)RR levels in women destined to deliver preterm may contribute to the development of kidney dysfunction that is common in women who birth preterm. Additionally, while third trimester s(P)RR levels tend to positively correlate with gestational age in women who birthed at term, s(P)RR levels do not correlate with gestational age in women who birthed preterm (Fig. 1*D*). Thus, s(P)RR levels may increase earlier in gestation in women who birth preterm.

This study is unique in its nature, as a biochemical study in a First Nations population. This study is limited however as it did not include samples from all gestations of pregnancy. This is important in order to determine the expression of s(P)RR across gestation and in the initial stages and/or early onset of pregnancy complications that arise before the third trimester (diabetes and preeclampsia). Additionally, while this study involved a large cohort of women carrying First Nation infants, the number of participants in our pregnancy outcome groups were low and this may have affected study results. It is important to note however that

the total number of participants in the larger Gomeroi Gaayggal Study is comparable with other First Nation studies of a similar nature [32].

### 4.1. Conclusion

To conclude, this is the first study that examines s(P)RR levels in pregnant women carrying First Nations babies. We have shown that s(P)RR is correlated with markers of kidney function and gestational age in this cohort. Additionally, we have shown that maternal plasma s(P)RR levels are not changed in preeclamptic/GDM or SGA/LGA pregnancies. Hence, s(P)RR may not be a suitable biomarker for these pathologies in First Nations women. Importantly, we have shown that maternal plasma s(P)RR levels tend to be increased during the third trimester in women who are destined to birth preterm. This suggests that s(P)RR may be a potential biomarker for preterm birth in this population although future studies in larger cohorts are required. Alternatively, s(P)RR may be a potential therapeutic target in the treatment of preterm birth-related kidney dysfunction.

## 5. Data sharing statement

The data presented in this study are available on request from the corresponding author and the Gomeroi Gaaynggal Advisory Committee. The data are not publicly available for ethical reasons.

## Authors contributions

SKE, ERL, KGP and The Gomeroi Gaaynggal Advisory Committee made substantial contributions to the conception and design of this manuscript. Those listed in the acknowledgements section below and RGS made substantial contributions to data acquisition. SKE, CB, OMO and KGP made substantial contributions to the analysis of data in this manuscript. SKE, CB, ERL, KGP and the Gomeroi Gaaynggal Advisory Committee were integral to the interpretation of data within this manuscript. SKE drafted this manuscript. SKE, KMR, ERL, KGP and the Gomeroi Gaaynggal Advisory Committee revised this manuscript. All authors read and approved the final manuscript and accept responsibility for the paper as published.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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