



Reference intervals for plasma renin and aldosterone concentrations in healthy normotensive multi-ethnic Malaysian adults using DiaSorin chemiluminescent immunoassay

Siow Phing Tay^{a,1}, Huai Heng Loh^{a,b,1,*}, Arlizan Baizura Ariffin^a, Anselm Ting Su^a, Nurul Farhana Satar^a, Ttarcini Engatramana^c, Mohammad Izzad Ishak^c, Simon Heng Haut Su^c, Liwan Lasem^a, Norliza Samsuri^a, Chee Shee Chai^a, Mohammad Masrin Md Zahrin^c, Jun Jun Thien^c, Xun Ting Tiong^c, Florence Hui Sieng Tan^c, Elena Aisha Azizan^b, Norlela Sukor^{b,d}

^a Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak (UNIMAS), Sarawak, Malaysia

^b Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia

^c Sarawak General Hospital, Sarawak, Ministry of Health Malaysia, Malaysia

^d Department of Medicine, Hospital Canselor Tuanku Muhriz, Kuala Lumpur, Malaysia

ARTICLE INFO

Keywords:

Population-specific

Renin-angiotensin-aldosterone system

Southeast Asia

ABSTRACT

Introduction: Accurate interpretation of plasma renin concentration (PRC) and plasma aldosterone concentration (PAC) requires population-specific reference intervals, which remain scarce in Southeast Asian populations. Manufacturer-derived reference intervals are based predominantly on Western cohorts and may not be directly applicable to regional populations.

Materials and methods: This cross-sectional study recruited 300 healthy, normotensive, non-obese adults (149 males, 151 females; mean age 34.4 ± 10.7 years) from a multi-ethnic Malaysian cohort in Sarawak to establish reference intervals for PAC and PRC. Strict exclusion criteria were applied to minimize confounding from medications, smoking, obesity, renal dysfunction, and hypokalemia. PAC and PRC were measured under standardized seated morning conditions using the DiaSorin Liaison® XL chemiluminescent immunoassay (CLIA). Reference intervals were derived using the IFCC-recommended non-parametric method, with sex- and age-stratified analyses.

Results: The reference intervals were 2.13–30.1 ng/dL (2.5th to 97.5th percentile) for PAC (range 1.25–42.30 ng/dL) and 7.4–66.4 µIU/mL (5th to 95th percentile) for PRC (range 1.31–107.80 µIU/mL). Participants aged ≥ 40 years had significantly lower PAC and PRC than younger participants. On multivariable regression analysis, PAC was independently associated with serum potassium and estimated 24-h urine sodium excretion, whereas PRC was independently associated with age, sex, systolic blood pressure, and estimated 24-h urinary sodium excretion. No significant difference in PAC and PRC was observed across ethnic groups.

Conclusions: We report the first platform-specific, sex- and age-stratified reference intervals for PAC and PRC in a healthy Southeast Asian population using the DiaSorin Liaison® XL CLIA platform. The derived intervals differed

Abbreviations: BMI, Body mass index; BP, Blood pressure; CLIA, Chemiluminescence immunoassay; CLSI, Clinical and Laboratory Standards Institute; CV, Coefficients of variation; eGFR, Estimated glomerular filtration rate; IQR, Interquartile range; LC-MS/MS, Liquid chromatography-tandem mass spectrometry; NSAID, Non-steroidal anti-inflammatory drug; PA, Primary aldosteronism; PAC, Plasma aldosterone concentration; PRC, Plasma renin concentration; RAAS, Renin-angiotensin-aldosterone system; RIA, Radioimmunoassay; RLU, Relative light units.

* Corresponding author at: Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak (UNIMAS), Sarawak, Malaysia.

E-mail addresses: sptay@unimas.my (S.P. Tay), hhloh@unimas.my (H.H. Loh), aabaizura@unimas.my (A.B. Ariffin), stanselm@unimas.my (A.T. Su), farnurul30@gmail.com (N.F. Satar), ttarchu@gmail.com (T. Engatramana), izzadishak90@yahoo.com (M.I. Ishak), simonsu@moh.gov.my (S.H.H. Su), liwan@unimas.my (L. Lasem), snorliza@unimas.my (N. Samsuri), cschai@unimas.my (C.S. Chai), masrin.zahrin@moh.gov.my (M.M.M. Zahrin), thienjunjun@yahoo.com (J.J. Thien), tiongxt.crc@gmail.com (X.T. Tiong), tanhuisieng@moh.gov.my (F.H.S. Tan), elena.azizan@ukm.edu.my (E.A. Azizan), drlela@hctm.ukm.edu.my (N. Sukor).

¹ Joint first authors

<https://doi.org/10.1016/j.clinbiochem.2026.111175>

Received 9 June 2026; Received in revised form 4 July 2026; Accepted 22 July 2026

Available online 25 July 2026

0009-9120/© 2026 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

from manufacturer-provided values, highlighting the importance of locally validated reference data for accurate interpretation of RAAS biomarkers and clinical evaluation of endocrine and hypertensive disorders.

1. Introduction

The renin-angiotensin-aldosterone system (RAAS) is a key regulator of blood pressure (BP), sodium balance, and fluid homeostasis. In clinical practice, plasma renin concentration (PRC) and plasma aldosterone concentration (PAC) are commonly measured to assess RAAS activity in a range of disorders, including primary aldosteronism (PA), secondary hypertension, and adrenal insufficiency. Accurate interpretation of PRC and PAC requires population-specific reference intervals, which may be influenced by age, sex, ethnicity, environmental factors, and analytical methodology.

International laboratory guidelines recommend that clinical laboratories establish or verify local applicable reference intervals whenever feasible, particularly when population characteristics differ from those used in manufacturer studies [1]. However, manufacturer-provided reference intervals are frequently derived from relatively small or demographically restricted populations and may not adequately reflect regional biological diversity. Variations in ethnicity, environmental exposures, dietary habits, and analytical platforms may all influence circulating concentrations of RAAS biomarkers [2].

Despite these recommendations, data on reference intervals for RAAS biomarkers in Southeast Asian populations remain limited. Most published reference interval studies have been conducted in Western populations and have primarily included White, Black, and Hispanic individuals. Ethnic differences in RAAS activity have been reported, with Black individuals generally exhibiting lower aldosterone concentrations and Hispanics demonstrating higher plasma renin activity (PRA) than White populations [3]. Furthermore, although radioimmunoassay (RIA), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and automated chemiluminescence immunoassay (CLIA) methods show reasonable concordance for screening purposes [4–6], absolute aldosterone and renin concentrations may differ substantially between analytical platforms [7,8]. Consequently, platform-specific reference intervals are required for accurate clinical interpretation.

Automated CLIA platforms, such as the DiaSorin Liaison® XL system, have increasingly replaced conventional RIAs in routine clinical laboratories because of their automation, rapid turnaround times, and avoidance of radioactive materials [9]. However, the manufacturer-derived reference intervals established for the DiaSorin Liaison® XL system were established from a cohort of only 89 and 126 individuals for PRC and PAC respectively, comprising Caucasian, African American, Hispanic, and Asian participants sampled in the upright and supine positions. These conditions differ from seated sampling protocols commonly used in clinical practice and may not adequately reflect the demographic characteristics of Southeast Asian populations.

The present study was therefore undertaken to establish platform-specific reference intervals for PRC and PAC using the DiaSorin Liaison® XL CLIA system in a well-characterized cohort of healthy normotensive adults from Sarawak, Malaysia, a multi-ethnic population comprising Malay, Chinese, and indigenous Sarawakian communities (including Iban and Bidayuh). Measurements were performed under standardized pre-analytical conditions, and sex- and age-stratified analyses were conducted. In addition, independent demographic and clinical determinants of PRC and PAC were evaluated using multivariable regression analysis.

2. Materials and methods

2.1. Study participants

This cross-sectional study was conducted at Sarawak General

Hospital, Kuching, Sarawak, Malaysia, between December 2021 and December 2022. Participants were recruited from healthy blood donors attending the hospital Blood Bank, who were considered broadly representative of the multi-ethnic adult population of Kuching. The minimum sample size of 120 individuals per sex partition was determined in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines EP28-A3C for non-parametric reference interval estimation [10].

The inclusion criteria were Malaysians aged ≥ 18 years, body mass index (BMI) < 27.5 kg/m² (based on the World Health Organization Asian-specific obesity threshold) [11], and estimated glomerular filtration rate (eGFR) > 60 mL/min/1.73m². Exclusion criteria included current smoking; pregnancy; hypertension (defined as systolic BP > 140 mmHg and/or diastolic BP > 90 mmHg) or use of antihypertensive medications; hypokalemia (serum potassium < 3.5 mmol/L); and use of medications known to affect the RAAS [12], including non-steroidal anti-inflammatory drugs (NSAIDs) and exogenous estrogens (oral contraceptive pills or hormone replacement therapy) within three months prior to sampling.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Medical Research Ethics Committee of Malaysia (NMRR-21-1874-61479). All participants provided written informed consent.

2.2. Socio-demographic and anthropometric data collection

Demographic data, including age, sex, ethnicity, medical history, and family history of hypertension, were collected through structured interviews. Height and weight were measured using standardized procedures, and BMI was calculated as weight (kg) divided by height squared (m²).

BP measurements were obtained between 08:00 and 11:00 h after participants had rested in the seated positions for at least 5 min. Measurements were performed using a validated automated sphygmomanometer (Omron HEM-7130-L, Omron Healthcare) on the right arm. The mean of two readings taken 1–2 min apart was used for analysis, in accordance with European Society of Hypertension recommendations [13].

2.3. Blood and urine sample collection

Participants remained seated for at least 15 min before venipuncture. Blood samples were collected between 08:00 and 11:00 h under standardized conditions. A total of 10 mL of venous blood was obtained by antecubital venipuncture into K₂-EDTA tubes (Becton Dickinson, USA) for PAC and PRC measurements and plain tubes (Becton Dickinson, USA) for serum electrolytes and creatinine.

Renin is susceptible to cryoactivation, whereby cold exposure of whole blood or plasma promotes conversion of inactive prorenin to active renin, potentially causing spurious elevation of measured PRC or PRA [14]. To minimize this risk, blood samples were maintained at room temperature (27–30 °C) during transport and were centrifuged within 30 min of collection at 3000 rpm (1734 x g) for 10 min. Plasma aliquots were subsequently stored at -80 °C until batch analysis, consistent with published recommendations demonstrating the stability of renin under these conditions [15]. Serum electrolytes and creatinine were measured within 2 h of collection using a Roche Cobas® c702 chemistry analyzer (Roche Diagnostics).

Spot urine samples were collected to estimate habitual dietary sodium intake using the INTERSALT equation. Estimated 24-h urinary sodium excretion was converted to estimated daily salt intake by

multiplying by 2.54 [16,17]. Renal function was assessed using the CKD-EPI 2021 equation. No dietary sodium restriction was imposed before sampling to reflect routine clinical practice.

2.4. PAC and PRC measurement

PAC and PRC were measured using the DiaSorin Liaison® XL fully automated CLIA platform (DiaSorin S.p.A., Saluggia, Italy). PAC was determined using the Liaison® Aldosterone Assay, whereas PRC was measured using the Liaison® Direct Renin Assay.

The aldosterone assay is a competitive immunoassay in which aldosterone present in the sample competes with an isoluminol-labelled aldosterone conjugate for binding to a sheep anti-aldosterone monoclonal antibody. The resulting chemiluminescent signal measured by the system photomultiplier as relative light units (RLU) is inversely proportional to the aldosterone concentration. The analytical measurement range was 0.97–100 ng/dL.

The direct renin assay is a two-site sandwich immunoassay employing monoclonal antibodies directed against renin and prorenin molecules. The generated chemiluminescent signal is directly proportional to the concentration of renin in the sample. The analytical measurement range was 0.52–500 μ IU/mL.

Analytical precision was verified according to CLSI EP15-A2 recommendations and met the manufacturer's performance specifications. For PAC, intra-assay and inter-assay coefficients of variation (CVs) were 2.6% and 5.9% at 7.73 ng/dL, and 1.5% and 3.9% at 29.4 ng/dL, respectively. For PRC, the corresponding intra- and inter-assay CVs were 2.8% and 4.1% at 23.5 μ IU/mL, and 2.4% and 4.1% at 115.77 μ IU/mL, respectively.

2.5. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Data distributions were assessed using histograms, boxplots, and density plots. Outliers were identified using the D/R ratio method for non-parametric reference interval estimation, as per CLSI EP28-A3C guideline [10].

The distribution of continuous variables was assessed using the Shapiro-Wilk test and visual inspection of histograms. Group comparisons were performed using the Welch's *t*-test for normally distributed variables whereas the Mann-Whitney *U* test for non-normally distributed variables. Associations between categorical variables were assessed using the chi-square tests.

Reference intervals were established using the non-parametric percentile method recommended by IFCC/CLSI EP28-A3C, corresponding to the 2.5th and 97.5th percentiles of the reference distribution, with 90% confidence intervals calculated. A log-normal parametric approach was additionally applied to evaluate consistency of the derived intervals. Sex- and age-specific reference intervals were calculated for all analytes. Age partitioning was undertaken a priori using a threshold of 40 years based on Malaysian epidemiological data demonstrating a marked increase in hypertension prevalence beyond this age [18]. To control for the confounding effect of age when comparing PAC and PRC between sexes, an age-matched cohort was generated using the case control matching procedure in IBM SPSS statistics. Male and female participants were matched 1:1 by age, and successful matching was confirmed by the absence of a significant difference in mean age between the two groups ($p = 1.0$). Comparisons of PAC and PRC between the age-matched male and female groups were subsequently performed as described above.

Independent predictors of log-transformed PAC and PRC were evaluated using multivariable linear regression models including age, sex, ethnicity, systolic BP, diastolic BP, BMI, serum potassium, and estimated 24-h urinary sodium excretion as covariates. Statistical significance was defined as a two-sided *p* value of <0.05 . Results are expressed as mean $\pm$ SD or median (IQR) as appropriate, with regression coefficients

reported alongside 95% confidence intervals.

3. Results

3.1. Participant characteristics

A total of 740 consecutive healthy adults were screened, of whom 309 participants fulfilled the eligibility criteria and completed study procedures. Seven participants were subsequently excluded because of hypokalemia, and two were excluded as statistical outlier based on D/R ratio testing (PRC values of 200.20 μ IU/mL and 199.8 μ IU/mL), resulting in a final analytical cohort of 300 participants (149 males, 151 females; mean age 34.4 ± 10.7 years).

The cohort was ethnically diverse, comprising Chinese (39.3%), Malay (26.0%), indigenous Sarawakian groups (20.7%), and participants of mixed or other ethnic backgrounds (14.0%). Six female participants were post-menopausal. Baseline demographics, clinical, and biochemical characteristics are summarized in Table 1.

Male participants were significantly older and had higher BMI, systolic BP, diastolic BP, and estimated 24-h urinary sodium excretion than female participants (all $p < 0.001$). Median PAC was significantly lower in males than females, whereas median PRC did not differ significantly between sexes.

3.2. Reference intervals

The range for PAC was 1.25–42.30 ng/dL, while that for PRC was 1.31–107.80 μ IU/mL in the whole cohort. In comparison with the manufacturer's proposed reference intervals measured from EDTA-plasma for PAC of 2.21–35.3 ng/dL and 1.17–23.6 ng/dL (2.5th to 97.5th percentile; upright and supine postures respectively, $n = 126$) and for PRC of 4.4–46.1 μ IU/mL and 2.8–39.9 μ IU/mL (5th to 95th percentile; upright and supine postures respectively, $n = 89$), the reference intervals established in our cohort were 2.13–30.1 ng/dL (2.5th to 97.5th percentile) for PAC and 7.4–66.4 μ IU/mL (5th to 95th

Table 1
Sociodemographic, anthropometric, and laboratory data of study participants.

	All cohort <i>n</i> = 300	Male <i>n</i> = 149	Female <i>n</i> = 151	<i>p</i>
Age, years	34.4 $\pm$ 10.7	37.5 $\pm$ 11.0	31.4 $\pm$ 9.6	<0.001
Ethnicity, <i>n</i> (%)				0.021
Chinese	118 (39.3)	68 (45.6)	50 (33.1)	
Malay	78 (26.0)	43 (28.9)	35 (23.2)	
Sarawak Indigenous	62 (20.7)	24 (16.1)	38 (25.2)	
Mixed heritage	25 (8.3)	9 (6.0)	16 (10.6)	
Others	17 (5.7)	5 (3.4)	12 (7.9)	
Family history of HPT, <i>n</i> (%)	87 (29)	47 (31.5)	40 (26.5)	0.335
Body mass index, kg/m ²	23.1 $\pm$ 2.5	23.7 $\pm$ 2.4	22.6 $\pm$ 2.5	<0.001
Systolic BP, mmHg	120.4 $\pm$ 11.3	126.1 $\pm$ 8.7	114.7 $\pm$ 10.8	<0.001
Diastolic BP, mmHg	77.4 $\pm$ 6.9	79.5 $\pm$ 6.2	75.2 $\pm$ 7.0	<0.001
Serum potassium, mmol/L	4.1 $\pm$ 0.3	4.1 $\pm$ 0.3	4.1 $\pm$ 0.3	0.181
eGFR, ml/min/1.73m ²	97.3 $\pm$ 18.9	95.6 $\pm$ 19.1	99.0 $\pm$ 18.6	0.118
Estimated urine Na, mmol/d	130.4 $\pm$ 33.5	148.1 $\pm$ 33.5	112.8 $\pm$ 22.6	<0.001
Estimated salt intake, g/d	7.7 $\pm$ 1.9	8.7 $\pm$ 1.8	6.6 $\pm$ 1.3	<0.001

Numerical variables are presented as the mean $\pm$ standard deviation while categorical variables are defined as absolute number and percentage. The *p* values were obtained from independent *t*-test (for normally distributed data) and chi-square test (for categorical data).

BP: blood pressure; eGFR: estimated glomerular filtration rate; HPT: hypertension; Na: sodium.

percentile) for PRC.

Sex-stratified analysis demonstrated significantly higher median PAC in females [9.27 ng/dL (IQR 6.49–15.00)] than in males [6.67 ng/dL (IQR 5.04–9.55); $p < 0.001$] (Table 2a). In contrast, median PRC did not differ significantly between sexes ($p = 0.300$). The sex-related difference in PAC remained significant even after exclusion of postmenopausal women and following age matching of male and female participants (Table 2b).

Age-stratified analyses demonstrated significantly lower median PAC ($p = 0.033$) and PRC ($p < 0.001$) among participants aged ≥ 40 years compared with those aged < 40 years (Table 3a). These findings were observed consistently in both sexes (Table 3b). Nevertheless, the wide and disparate IQR between the two age groups among the female participants suggest caution in interpreting this finding, given the relatively small sample size in the older female subgroup ($n = 33$).

No statistically significant differences in PAC or PRC were observed across ethnic groups ($p = 0.091$ and $p = 0.364$ respectively) (Supplementary Table 1).

3.3. Predictors of PAC and PRC

Multivariable regression analyses are summarized in Table 4. PAC was independently associated with serum potassium concentration ($\beta = 0.143$, $p = 0.009$) and estimated 24-h urinary sodium excretion ($\beta = -0.300$, $p < 0.001$).

PRC was independently associated with age ($\beta = -0.262$, $p < 0.001$), sex ($\beta = -0.197$, $p < 0.001$), systolic BP ($\beta = -0.173$, $p = 0.043$), and estimated 24-h urinary sodium excretion ($\beta = -0.243$, $p < 0.001$).

Neither BMI nor ethnicity was independently associated with PAC or PRC after adjustment for covariates.

4. Discussion

This study establishes the first platform-specific reference intervals for PAC and PRC in a healthy normotensive Southeast Asian adult population, derived under standardized pre-analytical conditions using the DiaSorin Liaison® XL CLIA platform. The derived reference intervals differed from the manufacturer-provided values, particularly for PRC, and those reported in other populations. These findings underscore the importance of establishing population-specific reference intervals and provide a valuable reference dataset for the accurate clinical interpretation of RAAS biomarkers in this region.

The differences observed between our reference intervals and those provided by the assay manufacturer are likely attributable to both biological and methodological factors. The manufacturer-derived reference intervals were established in a cohort of only 89 and 126 individuals respectively comprising predominantly Western populations sampled in the upright and supine positions. In contrast, our study employed standardized seated sampling following a defined rest period in a Southeast Asian population. Given the well-established influence of posture on RAAS activity [19], differences in sampling position may have contributed substantially to the observed discrepancies. Furthermore, differences in genetic background, environmental exposures, dietary habits, and population characteristics may also influence circulating renin and aldosterone concentrations [3,20,21].

Physiologically, higher sodium intake suppresses renin and aldosterone secretion, whereas lower sodium intake stimulates RAAS activation [22]. Consistent with this biological relationship, estimated 24-h urinary sodium excretion emerged as an independent predictor of both PAC

Table 2a
Comparison of PAC and PRC between sexes of study participants.

Parameters	Males $n = 149$	Females $n = 151$	p
PAC, ng/dL	6.67 (5.04, 9.55)	9.27 (6.49, 15.00)	<0.001
PRC, $\mu\text{IU/mL}$	24.24 (15.75, 33.36)	25.88 (14.65, 39.36)	0.300

Table 2b
Comparison of PAC and PRC between sexes matched for age, with exclusion of postmenopausal women.

Parameters	Males $n = 99$	Females $n = 99$	p
Age, years	33.8 ± 8.8	33.8 ± 8.8	1.0
PAC, ng/dL	6.70 (5.15, 9.82)	9.06 (6.54, 13.20)	<0.001
PRC, $\mu\text{IU/mL}$	26.80 (17.20, 33.45)	22.06 (14.20, 35.66)	0.396

Numerical variables are presented as the median (IQR 25th to 75th percentile). The p -values were obtained from Mann-Whitney test.

PAC = plasma aldosterone concentration; PRC = plasma renin concentration.

Table 3a
Comparison of PAC and PRC between age groups.

Parameters	Age < 40 years old $n = 201$	Age ≥ 40 years old $n = 99$	p
PAC, ng/dL	8.34 (5.75, 12.50)	6.99 (5.20, 9.86)	0.033
PRC, $\mu\text{IU/mL}$	27.67 (17.39, 42.54)	19.99 (12.5, 29.91)	<0.001

Table 3b
Comparison of PAC and PRC between age groups, stratified by sex.

Parameters	Males		p	Females		p
	Age < 40 years $n = 83$	Age ≥ 40 years $n = 66$		Age < 40 years $n = 118$	Age ≥ 40 years $n = 33$	
PAC, ng/dL	7.24 (5.08, 9.90)	6.50 (5.01, 9.19)	0.354	9.29 (6.38, 16.10)	7.85 (6.28, 12.05)	0.481
PRC, $\mu\text{IU/mL}$	26.89 (17.68, 36.80)	20.4 (14.5, 30.3)	0.026	28.90 (16.68, 46.37)	14.99 (9.13, 26.72)	<0.001

Numerical variables are presented as the median (IQR 25th to 75th percentile). The p -values were obtained from Mann-Whitney test.

PAC = plasma aldosterone concentration; PRC = plasma renin concentration.

Table 4
Multivariable linear regression analysis of log PAC and log PRC.

Predictors	β	95% CI	p
Dependent variable: Log PAC			
Adjusted $R^2 = 0.173$			
Age	0.024	-0.002, 0.004	0.693
Sex	0.095	-0.022, 0.124	0.173
Ethnicity	0.014	-0.021, 0.028	0.258
Systolic BP	-0.090	-0.006, 0.002	0.288
Diastolic BP	0.071	-0.003, 0.008	0.335
Body mass index	0.013	-0.012, 0.015	0.841
Serum potassium	0.143	0.029, 0.201	0.009
24-h urine sodium excretion	-0.300	-0.004, -0.001	<0.001
Dependent variable: Log PRC			
Adjusted $R^2 = 0.142$			
Age	-0.262	-0.011, -0.004	<0.001
Sex	-0.197	-0.200, -0.036	<0.001
Ethnicity	0.010	-0.025, 0.030	0.862
Systolic BP	-0.173	-0.009, 0	0.043
Diastolic BP	0.089	-0.002, 0.010	0.229
Body mass index	-0.003	-0.015, 0.015	0.957
Serum potassium	-0.010	-0.106, 0.087	0.850
24-h urine sodium excretion	-0.243	-0.003, -0.001	<0.001

BP: blood pressure; CI: confidence interval; PAC: plasma aldosterone concentration; PRC: plasma renin concentration.

and PRC in our cohort. Interestingly, despite a lower estimated daily salt intake than that reported in Chinese populations [23,24], median PAC and PRC in our cohort were comparable to those reported by Deng et al. [25], who used the same analytical platform and similar seated sampling conditions. This observation further highlights the complexity of factors influencing RAAS activity and underscores the importance of

establishing population-specific reference intervals.

Although no statistically significant differences in PAC or PRC were observed among ethnic groups in our cohort, the sample sizes of individual ethnic subgroups may have limited the statistical power to detect modest biological differences. Larger studies will be required to determine whether ethnicity-specific reference intervals are warranted within Southeast Asian populations. Nevertheless, previous studies have demonstrated ethnic differences in PAC and PRA among White, Hispanic, and Chinese populations [3], supporting the biological plausibility of population-specific RAAS profiles. It is also noteworthy that many earlier studies did not explicitly exclude smokers or users of oral contraceptive pills. Nicotine has been shown to activate RAAS and increase aldosterone concentrations [26], whereas oral contraceptive use may increase PAC while suppressing PRC [27], potentially contributing to variability between studies.

The age-related decline in PRC and PAC observed in our study is consistent with findings reported across multiple populations and analytical platforms [25,28–30]. Age-related alterations in adrenal aldosterone synthase expression, reduced adrenal responsiveness to angiotensin II, and progressive decline in juxtaglomerular cell function associated with nephron loss are thought to contribute to reduced RAAS activity with aging [31,32]. Previous studies have demonstrated a gradual decline in renin and aldosterone concentrations across adulthood [33]. This pattern was evident in the overall cohort and was particularly pronounced among male participants. Among women, PRC was significantly lower in those aged ≥ 40 years than in those < 40 years, whereas PAC was numerically lower but did not reach statistical significance. However, the variability of PAC and PRC differed substantially between the two female age groups, likely because of the relatively small number of women aged ≥ 40 years ($n = 33$), thereby limiting the statistical power. Our findings nonetheless confirm that these age-related patterns are also present in a Southeast Asian population and support consideration of age-specific reference intervals when interpreting RAAS biomarkers in clinical practice.

We also observed significantly higher PAC in females than in males, even after exclusion of post-menopausal women and after age-matching. This finding is consistent with previous reports [20,24,25,30,34]. Estrogen influences multiple components of the RAAS, including substrate availability, enzyme activity, receptor expression, and protein synthesis [35], which may contribute to higher aldosterone concentrations without a corresponding rise in PRC. Although menstrual cycle phase was not standardized in this study, the persistence of the observed sex differences following age-matching and exclusion of post-menopausal women suggests that the findings are robust.

The DiaSorin Liaison® XL CLIA was selected because it is widely used in routine clinical laboratories for the measurement of aldosterone and renin. Although LC-MS/MS remains the reference analytical method for steroid hormone measurement, its technical complexity and operational costs limit widespread implementation in many Southeast Asian laboratories [36]. Consequently, the reference intervals established in this study are directly applicable to clinical laboratories utilizing the DiaSorin Liaison® XL platform.

The clinical implications of these findings are important. Application of manufacturer-derived reference intervals established predominantly in Western populations may result in misclassification of normal renin or aldosterone concentrations in Southeast Asian individuals. The availability of locally derived reference intervals may therefore improve interpretation of RAAS biomarkers and enhance diagnostic accuracy during the evaluation of endocrine hypertension, PA, and disorders of adrenal function.

Several limitations should be acknowledged. First, menstrual cycle phase was not standardized among pre-menopausal women, which may have contributed to variability in PAC measurements. However, this reflects routine clinical practice and is consistent with previously published RAAS reference interval studies [34,37]. Second, this was a single-center study conducted in Kuching, Sarawak, which may limit

generalizability to other regions of Malaysia and Southeast Asia. Third, dietary sodium intake was estimated using a single spot urine sample and the INTERSALT equation rather than direct 24-h urine collection. Although validated for population-level assessment, this approach may introduce individual-level variability in sodium estimation. In addition, despite inclusion of multiple ethnic groups, the sample sizes of individual ethnic subgroups may have been insufficient to detect modest ethnic differences in RAAS biomarkers. Finally, the reference population was derived from blood donors rather than the general healthy population. Although blood donors are generally considered healthy individuals, they may not be fully representative of the broader population because of potential differences in sociodemographic characteristics, lifestyle factors, and occupation. Therefore, the reference intervals established in this study may not be entirely generalizable to broader healthy adult population.

Nevertheless, this study has several notable strengths, including rigorous exclusion of major RAAS confounders, standardized pre-analytical sampling procedures, use of a widely adopted automated analytical platform, and recruitment of a multi-ethnic cohort exceeding the CLSI-recommended sample size requirements for sex-specific reference interval estimation. These methodological strengths enhance the robustness and clinical applicability of the reference intervals derived.

5. Conclusions

This study establishes the first platform-specific reference intervals for PAC and PRC in a healthy, normotensive, multi-ethnic Southeast Asian population using the DiaSorin Liaison® XL CLIA platform under standardized seated morning sampling conditions. The derived reference intervals differ from manufacturer-provided values, highlighting the importance of locally validated reference data for accurate interpretation of RAAS biomarkers and reducing the risk of diagnostic misclassification in clinical practice.

Significant age- and sex-related differences in PAC and PRC were also observed, suggesting that these demographic factors should be considered when interpreting RAAS biomarker results. The reference intervals generated in this study provide a clinically relevant framework for laboratories and clinicians using the DiaSorin Liaison® XL platform in Southeast Asian populations.

Future multicenter studies involving larger and more diverse populations, standardized sodium balance protocols, and parallel LC-MS/MS validation are warranted to further refine reference intervals, improve analytical harmonization, and understanding of physiological variation in RAAS biomarkers across Southeast Asian populations.

CRedit authorship contribution statement

Siow Phing Tay: Writing – review & editing, Resources, Methodology, Investigation, Data curation, Conceptualization. **Huai Heng Loh:** Writing – original draft, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Arlizan Baizura Ariffin:** Writing – review & editing, Validation. **Anselm Ting Su:** Formal analysis. **Nurul Farhana Satar:** Data curation. **Ttarcini Engatramana:** Data curation. **Mohammad Izzad Ishak:** Data curation. **Simon Heng Haut Su:** Data curation. **Liwan Lasem:** Data curation. **Norliza Samsuri:** Data curation. **Chee Shee Chai:** Resources. **Mohammad Masrin Md Zahrin:** Resources. **Jun Jun Thien:** Validation, Resources. **Xun Ting Tiong:** Resources. **Florence Hui Sieng Tan:** Supervision. **Elena Aisha Azizan:** Writing – review & editing, Supervision. **Norlela Sukor:** Writing – review & editing, Supervision, Methodology.

Funding

Universiti Malaysia Sarawak (UNIMAS) provided financial support in the form of UNIMAS PILOT Research Grant (UNI/F05/PILOT/85091/2022). The sponsors had no role in the design or conduct of this research

paper.

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.

Acknowledgments

The authors would like to thank Dr. Nur Ain Zunaidi, Dr. Siew Ling Lu, and Dr. Gong Quan Lau for their contribution in donor screening, and Ms. Maria Mei Fung Jong, Ms. Siew Siew Wong, and Ms. Mimipariza Jemulidin for their assistance in sample processing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clinbiochem.2026.111175>.

References

- [1] Y. Ozarda, Reference intervals: current status, recent developments and future considerations, *Biochem Med (Zagreb)* 26 (1) (2016) 5–16.
- [2] P.M. O'Shea, T.P. Griffin, S. Denieffe, M.C. Fitzgibbon, The aldosterone to renin ratio in the diagnosis of primary aldosteronism: promises and challenges, *Int. J. Clin. Pract.* 73 (7) (2019) e13353.
- [3] D.E. Rifkin, A.R. Khaki, N.S. Jenny, R.L. McClelland, M. Budoff, K. Watson, et al., Association of renin and aldosterone with ethnicity and blood pressure: the multi-ethnic study of atherosclerosis, *Am. J. Hypertens.* 27 (6) (2014) 801–810.
- [4] F. Pizzolo, G. Salvagno, B. Caruso, C. Cocco, F. Zorzi, C. Zaltron, et al., Fully automated chemiluminescence vs RIA aldosterone assay in primary aldosteronism work-up, *J. Hum. Hypertens.* 31 (12) (2017) 826–830.
- [5] M. Tetti, J. Burrello, J. Goi, M. Parasiliti-Capripino, G. Gioiello, F. Settanni, et al., Diagnostic accuracy of aldosterone and renin measurement by Chemiluminescence for screening of patients with primary Aldosteronism, *Int. J. Mol. Sci.* 25 (15) (2024).
- [6] W. Chen, F. Lai, X. Huang, S. Yu, N. Chen, C. Xu, et al., Comparison of plasma aldosterone measured by chemiluminescence immunoassay and liquid chromatography-tandem mass spectrometry in screening test for primary aldosteronism, *Practical Laboratory Medicine* 39 (2024) e00361.
- [7] Y. Taki, T. Kono, K. Teruyama, T. Ichijo, I. Sakuma, H. Nagano, et al., Comparative analysis of aldosterone and renin assays for primary aldosteronism screening, *Sci. Rep.* 14 (1) (2024) 26040.
- [8] Q. Zeng, J. Li, Y. Yang, Y. He, Y. Song, J. Hu, et al., Comparison of a chemiluminescence immunoassay with LC-MS/MS in the determination of the plasma aldosterone concentration in patients with impaired renal function, *Steroids* 213 (2025) 109540.
- [9] Y. Nam, H.D. Park, Revolutionizing laboratory practices: pioneering trends in Total Laboratory automation, *Ann. Lab. Med.* 45 (5) (2025) 472–483.
- [10] CLSI, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory Approved Guideline – CLSI – EP28-A3C 3rd edition, 28, Clinical and Laboratory Standards Institute, Wayne (PA), 2010.
- [11] W.H.O.E. Consultation, Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies, *Lancet* 363 (9403) (2004) 157–163.
- [12] G.K. Adler, M. Stowasser, R.R. Correa, N. Khan, G. Kline, M.J. McGowan, et al., Primary aldosteronism: an Endocrine Society clinical practice guideline, *J. Clin. Endocrinol. Metab.* 110 (9) (2025) 2453–2495.
- [13] G.S. Stergiou, P. Palatini, G. Parati, E. O'Brien, A. Januszewicz, E. Lurbe, et al., European Society of Hypertension practice guidelines for office and out-of-office blood pressure measurement, *J. Hypertens.* 39 (7) (2021).
- [14] S. Hepburn, C. Munday, K. Taylor, D.J. Halsall, Stability of direct renin concentration and plasma renin activity in EDTA whole blood and plasma at ambient and refrigerated temperatures from 0 to 72 hours, *Clin. Chem. Lab. Med.* 60 (9) (2022) 1384–1392.
- [15] P. Bonnitcha, M. Rigdwell, P. Ward, D. Cheshier, Standard –20°C freezer storage protocols may cause substantial plasma renin cryoactivation, *Clin. Chem. Lab. Med.* 61 (8) (2023) 1428–1435.
- [16] H.E. Meyer, L. Johansson, A.E. Eggen, H. Johansen, K. Holvik, Sodium and potassium intake assessed by spot and 24-h urine in the population-based Tromsø study 2015–2016, *Nutrients* 11 (7) (2019).
- [17] N. Qian, Y. Jiang, Y. Wang, P. Yan, F. Yao, M. Sun, et al., Validity of five formulas in estimating 24-h urinary sodium via spot urine sampling in hypertensive patients living in Northeast China, *J. Hypertens.* 39 (7) (2021) 1326–1332.
- [18] S.M. MJ CZ, Ching SM, Tan CH, Lee KW, Devaraj NK, Salim HS, Ramachandran V, Lim PY, Sivaratnam D, Hoo FK, Cheong AT, Chia YC. Prevalence, awareness and control of hypertension in Malaysia from 1980–2018: a systematic review and meta-analysis. *World J. Meta-Anal.* 2020;8(4):320–344.
- [19] M. Stowasser, A.H. Ahmed, E. Pimenta, P.J. Taylor, R.D. Gordon, Factors affecting the aldosterone/renin ratio, *Horm. Metab. Res.* 44 (3) (2012) 170–176.
- [20] C. Newton-Cheh, C.Y. Guo, P. Gona, M.G. Larson, E.J. Benjamin, T.J. Wang, et al., Clinical and genetic correlates of aldosterone-to-renin ratio and relations to blood pressure in a community sample, *Hypertension* 49 (4) (2007) 846–856.
- [21] S.J. Shin, C. Lim, S.W. Oh, M.Y. Rhee, The unique response of renin and aldosterone to dietary sodium intervention in sodium sensitivity, *J. Renin-Angiotensin-Aldosterone Syst.* 15 (2) (2014) 117–123.
- [22] W. Feng, Q. Cai, W. Yuan, Y. Liu, A.S.A. Bardeesi, J. Wang, et al., Low response of renin-angiotensin system to sodium intake intervention in Chinese hypertensive patients, *Medicine (Baltimore)* 95 (6) (2016) e2602.
- [23] J. Xu, J. Zhang, M. Liu, Y. Bai, X. Guo, J. Dong, et al., Estimating 24-hour sodium excretion from spot urine samples in Chinese adults: can spot urine substitute 24-hour urine samples? *Nutrients* 12 (3) (2020).
- [24] Y. Yin, C. Ma, S. Yu, Q. Ai, C. Han, J. Zhong, et al., Reference intervals for LC-MS/MS measurements of plasma renin activity, aldosterone, angiotensin II, and 24-hour urinary aldosterone in northern Chinese Han population, *Clin. Chim. Acta* 543 (2023) 117325.
- [25] L. Deng, Z. Xiong, H. Li, X. Lei, L. Cheng, Analytical validation and investigation on reference intervals of aldosterone and renin in Chinese Han population by using fully automated chemiluminescence immunoassays, *Clin. Biochem.* 56 (2018) 89–94.
- [26] J.M. Oakes, R.M. Fuchs, J.D. Gardner, E. Lazartigues, X. Yue, Nicotine and the renin-angiotensin system, *Am. J. Phys. Regul. Integr. Comp. Phys.* 315 (5) (2018) R895–R906.
- [27] A.H. Ahmed, R.D. Gordon, P.J. Taylor, G. Ward, E. Pimenta, M. Stowasser, Effect of contraceptives on aldosterone/renin ratio may vary according to the components of contraceptive, renin assay method, and possibly route of administration, *J. Clin. Endocrinol. Metab.* 96 (6) (2011) 1797–1804.
- [28] J.Y. Cheng, F.C. Wong, E.W. Chow, W.W. Lau, K.K. Cheung, T.H. Cheng, et al., Chinese normotensive and essential hypertensive reference intervals for plasma aldosterone and renin activity by liquid chromatography-tandem mass spectrometry, *Clin. Chem. Lab. Med.* 60 (10) (2022) 1640–1647.
- [29] A. Hannemann, N. Friedrich, J. Ludemann, H. Volzke, R. Rettig, J. Peters, et al., Reference intervals for aldosterone, renin, and the aldosterone-to-renin ratio in the population-based study of health in Pomerania (SHIP-1), *Horm. Metab. Res.* 42 (6) (2010) 392–399.
- [30] M.N. Kerstens, A.C. Kobold, M. Volmer, J. Koerts, W.J. Sluiter, R.P. Dullaart, Reference values for aldosterone-renin ratios in normotensive individuals and effect of changes in dietary sodium consumption, *Clin. Chem.* 57 (1611) (2011) 1607–1611.
- [31] K. Nanba, A. Vaidya, G.H. Williams, I. Zheng, T. Else, W.E. Rainey, Age-related autonomous Aldosteronism, *Circulation* 136 (4) (2017) 347–355.
- [32] R.H. Noth, M.N. Lassman, S.Y. Tan, A. Fernandez-Cruz Jr., P.J. Mulrow, Age and the renin-aldosterone system, *Arch. Intern. Med.* 137 (10) (1977) 1414–1417.
- [33] J.H. Bauer, Age-related changes in the renin-aldosterone system. Physiological effects and clinical implications, *Drugs Aging* 3 (3) (1993) 238–245.
- [34] P. O'Shea, J.J. Brady, N. Gallagher, M.C. Denny, M. Fitzgibbon, Establishment of reference intervals for aldosterone and renin in a Caucasian population using the newly developed immunodiagnostic systems specialty immunoassay automated system, *Ann. Clin. Biochem.* 53 (Pt 3) (2016) 390–398.
- [35] S.M. Nwia, A.P.O. Leite, X.C. Li, J.L. Zhuo, Sex differences in the renin-angiotensin-aldosterone system and its roles in hypertension, cardiovascular, and kidney diseases, *Front. Cardiovasc. Med.* 10 (2023) 1198090.
- [36] S.K. Grebe, R.J. Singh, LC-MS/MS in the clinical laboratory - where to from Here? *Clin. Biochem. Rev.* 32 (1) (2011) 5–31.
- [37] C. Zhang, Z. Guo, C. Yi, H. Wang, W. Zhang, Y. Zhao, Age- and sex-specific reference intervals for renin and aldosterone in healthy individuals in Yunnan Province, China, *J. Hum. Hypertens.* 39 (9) (2025) 652–657.