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Establishment of adult reference interval of oxalate in spot urine using real-world data

Ying Shen[1], Xia Luo[1], Qing Guan[1], Wenjie Lou[2*] and Liming Cheng[1*]

Abstract

Background Hyperoxaluria, characterized by excessive oxalate production, can be attributed to high dietary oxalate intake, genetic disorders affecting oxalate metabolism, or certain intestinal diseases. Despite its clinical significance, there is a paucity of comprehensive discussions regarding the reference interval (RI) for oxalate levels in spot urine samples. This study aims to establish an appropriate adult RI for oxalate in spot urine to enhance the interpretation of clinical data.

Methods Between January 2021 and July 2021, a cohort of 608 adults aged 19 to 85 years undergoing routine physical examinations was recruited to establish the RI. Additionally, 381 adults with complete datasets were analyzed to examine variables influencing the urinary oxalate/urinary creatinine (UOA/UCr) ratio. Urinary oxalate levels were quantified using a high-performance liquid chromatography assay assured by a proficiency testing from the College of American Pathologists.

Results The study established sex- and age-specific Ris for the UOA/UCr ratio. For males, three age-specific partitions were identified: 19–29 years (8.499–54.39 mmol/mol), 30–39 years (10.25–61.29 mmol/mol), and ≥ 40 years (12.13–68.69 mmol/mol). In contrast, for females, two age-specific partitions were required: 19–39 years (11.03–64.93 mmol/mol) and ≥ 40 years (11.00–93.84 mmol/mol). UALB/UCr and UREA were recognized as key confounding factors in linear regression that could account for the differences in the UOA/UCr ratio in people.

Conclusion Determining adult RI of oxalate in spot urine is crucial for diagnosing hyperoxaluria. Our sex and age-specific reference intervals will be beneficial for hyperoxaluria screening. The findings regarding the relationship between variables support explaining the variation of UOA/UCr ratio in individuals.

Keywords Oxalate, Spot urine, Reference interval, Adults

Background

Approximately 10–20% of the oxalate found in urine is from dietary oxalate that has been absorbed, with the rest coming from the body's metabolism of glyoxylate and ascorbic acid [1]. Oxalate could be secreted and absorbed by the gastrointestinal tract, and then almost entirely excreted by the kidney. The condition known as hyperoxaluria, characterized by increased oxalate in urine, can be attributed to dietary excess of oxalate or its precursors, inherited disorders of oxalate synthesis or excretion, and intestinal diseases [2]. Excessive oxalate production in the liver can cause increased kidney excretion leading to urolithiasis, nephrocalcinosis and eventually chronic

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kidney disease [3]. As a result, the assessment of oxalate is deemed significant. However, the proper interpretation of biomedical results is crucial for disease diagnosis and treatment [4]. Therefore, setting a suitable reference interval (RI) for oxalate is vital and essential for diagnosing hyperoxaluria.

Up to now, many studies have focused on determing the RI of oxalate in children for diagnosing primary hyperoxaluria (PH) [2, 5–8]. Nevertheless, it is well-known that there are three types of PH. PH type 1, the most frequent form, usually appears in childhood and affects 1 to 3 cases per million, while PH type 2 and type 3 can develop at all ages [3]. Thus, establishing the RI of oxalate in children is insufficient for diagnosing all types of PH or secondary hyperoxaluria in adults. Typically, the analysis of urinary excretion components has been conducted with urine gathered over a 24-h peroid. As an example, Zeng's group has already established the reference interval of oxalate in 24-h urine based on ion exchange chromatography [9]. However, the collection of 24-h urine is rather cumbersome. In comparison, the collection of spot urines is much simpler with good compliance. Within Elgstoen's study, a precursory adult reference interval for oxalate adjusted by creatinine in spot urines has been created based on 56 healthy individuals [10]. Yet, with a good level of interpretation, RI specific to a population could more effectively justify the disease. Therefore, determining the reference interval of oxalate in spot urine for Chinese adults with a substantial sample size is of great importance.

In our previous study, a high-performance liquid chromatography (HPLC) method has been successfully developed and the oxalate RI was established in children for hyperoxaluria screening [11]. Based on this well-validated method, this study will establish the adult RI for oxalate in spot urine and further explore variables that greatly affect individual variability.

Methods

Subjects

All apparently healthy subjects were enrolled from those who underwent physical examinations from January 2021 to July 2021 at Tongji hospital of Tongji Medical College of Huazhong University of Science and Technology. The inclusion criteria specified adults who are at least 18 years old. The exclusion criteria were that adults with renal diseases (kidney stone, hydronephrosis, nephritis, nephrotic syndrome and others), digestive system diseases (idiopathic inflammatory bowel disease, ileectomy, jejunal/ileal bypass, celiac disease, chronic pancreatitis, chronic biliary diseases, and unexplained diarrhea with abnormal fat absorption) and diabetes should be ruled out. The participants with renal diseases were initially excluded based on protein:creatinine ratio combined with creatinine itself as a simple and inexpensive procedure, while diabetes were excluded according to the WHO standard that fasting blood glucose levels $\geq$ 7.0 mmol/L. In addition, large doses (> 2 g orally/24 h) of vitamin C should be avoided before specimen collection. The basic demographic characteristics of participates including sex, age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP) and biochemical outcomes were collected through medical records, and complete data were available for 381 subjects. The ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology approved the study protocols and experiment was conducted in accordance with the Declaration of Helsinki.

The sample size was calculated using the formula $N=[Z_{1-\alpha/2}]^2\times P\ (1\text{-}P)/\mathrm{d}^2$ [12]. Where N is the sample size, $Z_{1-\alpha/2}$ (1.96) is the certainty wanted expressed in the percentage point of normal distribution corresponding to the 2-sided level of significant ($\alpha=0.05$); P (95%) is the estimated proportion of an attribute that is present in the population; d (2.0%) is the desired level of precision required for quantile estimation. Therefore, $N=[(1.96)^2\times 0.95\times(1-0.95)]/(0.02)^2=456$. A non response rate of 30% was added, giving a total sample of 592. Given the incomplete demographic data, a total sample of 610 was set to be included. Finally, a total of 608 samples were picked based on real-world scenarios. Meanwhile, a priori sampling involving establishment of clear exclusion and partitioning criteria beforehand sampling, and a stratified random sampling based on gender and age were used for choosing the reference individuals [12, 13]. Considering the practical aspect of collecting samples, 307 males and 301 females were finally selected in different age groups as shown in Table S1.

All participants collected early morning fasting urine samples. The spot urine samples were refrigerated as soon as possible and analyzed on the day of collection.

Measurements

The urinary oxalate concentrations were determined by a 1200 series HPLC system coupled with a ultraviolet detector (Agilent Technologies, Blackburn, Australia) based on our laboratory-developed LC method [11]. The samples were processed as follows: 1.0 mL of calibrators/quality controls/urine specimens were mixed with 5 μL of concentrated HCl (12 M) and 250 μL of OPD (0.46 M) solution in tubes. The tubes were capped, vortexed, and heated to 120 °C for 30 min. The solutions changed from yellow to dark brown. After the solutions were cooled, 30 μL of 10 M NaOH was added. Finally, the solutions were centrifuged for 10 min at 18,000 rpm for direct HPLC analysis. The concentrations of plasma or urine biochemical

outcomes, such as urinary albumin (UALB), urinary creatinine (UCr), plasma creatinine (Cr), urea (UREA), uric acid (UA) were measured on a Cobas 8000 system (Roche, Diagnostics, Germany). eGFR was calculated based on CKD-EPI creatinine Eq. (2009) as following [14]: Female (Scr ≤ 0.7 mg/dL): $eGFR = 144 \times (Scr/0.7)^{-0.329} \times 0.993^{Age}$; Female (Scr > 0.7 mg/dL): $eGFR = 144 \times (Scr/0.7)^{-1.209} \times 0.993^{Age}$; Male (Scr ≤ 0.9 mg/dL): $eGFR = 141 \times (Scr/0.9)^{-0.411} \times 0.993^{Age}$; Male (Scr > 0.9 mg/dL): $eGFR = 141 \times (Scr/0.9)^{-1.209} \times 0.993^{Age}$, where eGFR (estimated glomerular filtration rate) = mL/min/1.73 m^2, Scr = serum creatinine in mg/dL, Age (years).

External quality assessment

Urinary oxalate assay quality has been evaluated through proficiency testing (PT) by the College of American Pathologists (CAP). For CAP, three samples in each batch were sent every 6 months for testing and compared with other laboratories. Samples were shipped on dry ice and stored at − 80 °C until the specified data. Laboratories were encouraged to measure samples and were instructed to process the external quality control samples together with their own patient samples.

Statistical analysis

Data analysis for setting up RI were performed following the CLSI Guidelines C28-A3c [13]. A test proposed by Dixon was used to determine the outlines [15]. In Dixon method, it suggests that if the D/R is ≥ 1/3, the maximum or minimum value is estimated as an outline and the remaining values are used on the above steps until all outlines are excluded (R is the range of all detected results including extremes while D is the absolute difference between the largest or smallest result and the next largest or smallest result). After outlier exclusion, the Harris and Boyd method, as recommended by CLSI C28-A3c, was used to explore the need for sex and age partitioning. Initially, partitioning by sex was prior to age. Once deciding on sex-based partitioning, age-based partition would be assessed by starting with the youngest age group and adding the subsequent groups in order. In the Harris and Boyd method, the Z test and the Z^* test is calculated between subclasses as following:

$$Z = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n1} + \frac{s_2^2}{n2}}}, Z^* = 3\sqrt{\frac{n_1 + n_2}{240}}$$

where $\bar{x}_1$ and $\bar{x}_2$ represent the mean value from the two subgroups, s_1 and s_2 represent the standard deviations from the two subgroups, and n_1 and n_2 represent the sample size of the two subgroups. If the original data are high skewed, and a transform, such as the log transform, should be performed to produce a distribution of values much closer to Gaussian form which is preferable to apply the Z test. If Z exceeds Z^*, the two subgroups are recommended to be separated, or else they should be merged into one. It is noted that partitioning is suggested when the larger s, such as $s_2 \geq 1.5\ s_1$.

To assess data normality, the Kolmogorov–Smirnov test or Shapiro–Wilk tests were performed. Normally distributed data were expressed as mean ± SD, while non-normally distributed data were presented as median (Interquartile range, IQR). For all individiuals included, the RI for urinary oxalate/urinary creatinine (UOA/UCr) ratio was established as the central 95% ranging from 2.5 to 97.5th percentile. For normally distributed data, the differences in continuous variables between groups were tested by the Student's t test or the Analysis of Variance, while the Mann–Whitney U test or the Kruskal–Wallis test were used for data that was not normally distributed. Categorical data were analyzed using either Pearson's chi-square test or Fisher's exact test. The correlation strength between variables were assessed using Spearman's correlation.

The relationship of the UOA/UCr ratio with BMI, SBP, DBP, and biochemical indicators related to renal function were assessed using linear regression models. Both stepwise forward linear regression and backward elimination approaches were tested to determine the best overall model. For stepwise forward regression, the P-value to enter was defined as ≤ 0.05. Variables with a P-value ≤ 0.05 enter regression model one by one while those with a P-value above 0.05 are removed. For backward regression, the default P-value is 0.1 and each one is progressively removed if their P values are higher than 0.1. The final model by both methods was checked to confirm each variable made a significant contribution to UOA/UCr ratios. The variables with tolerance > 0.2 and variance inflation factor (VIF) < 2.0 indicates no significant multi-collinearity problems [16].

A P-value below 0.05 was considered statistically significant. Statistical analyses were done using SPSS (version 20.0; SPSS, Isnc., Chicago, Ill, USA) and the Medcalc version 11.4 (Medcalc, Ostend, Belgium). The correlation analysis heatmap was plotted by R language.

Results

The impact of age and sex on the reference intervals

In this study, a total of 608 participates (307 males and 301 females, 19 to 85 years old with a median age of 46) were enrolled to establish the LC assay-based RI of UOA/UCr ratio. Age differences between males and females were not statistically significant ($P = 0.673$) (Table S2). No outlines were identified by the Dixon ruler, and all data were included for RI establishment. The Kolmogorov–Smirnov test showed a skewed distribution for the

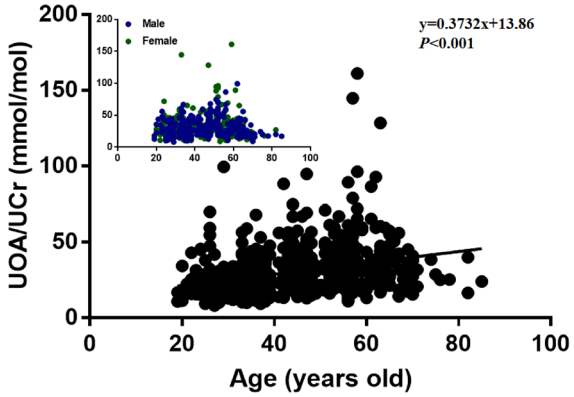

Fig. 1 The correlation between UOA/UCr ratios and age based on sex

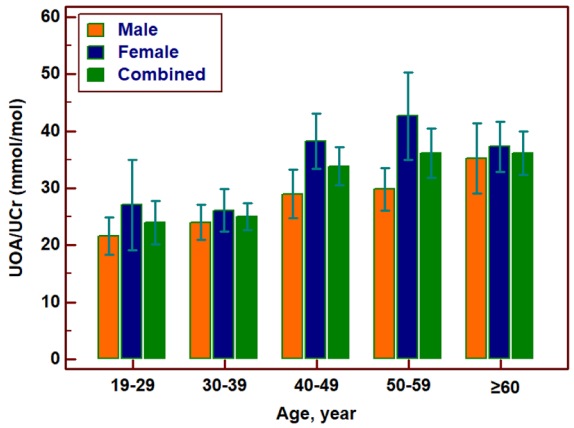

Fig. 2 The histograms distribution of average reference values for UOA/UCr ratios by age intervals and gender

Table 1 Group partitioning of RI for UOA/UCr ratio by sex and/or age[a]

Sex	*Z*	*Z**	Grouping
Male/female	6.29	4.77	Yes
Male			
Age (19–29 years/30–39 years)	2.26	2.16	Yes
Age (30–39 years/40–49 years)	5.35	2.27	Yes
Age (40–49 years/50–59 years)	1.57	2.25	No
Age (30–39 years/40–59 years)	3.66	2.83	Yes
Age (40–59 years/ ≥ 60 years)	1.34	2.61	No
Age (30–39 years/ ≥ 40 years)	4.17	3.12	Yes
Female			
Age (19–29 years/30–39 years)	0.57	2.16	No
Age (19–39 years/40–49 years)	6.27	2.66	Yes
Age (40–49 years/50–59 years)	1.57	2.25	No
Age (19–39 years/40–59 years)	8.04	3.12	Yes
Age (40–59 years/ ≥ 60 years)	0.03	2.58	No
Age (19–39 years/ ≥ 40 years)	8.54	3.38	Yes

[a] Data are log-transformed for normal distribution

Table 2 RI for UOA/UCr ratio ($n = 608$)[a]

Sex	*N*	Min.	Max.	Median (IQR)	RI (2.5–97.5th)
Male					
19–29	47	8.10	56.09	23.18 (15.85–34.54)	8.499–54.39
30–39	78	9.96	66.85	24.28 (17.25–37.61)	10.25–61.29
≥ 40	182	9.20	99.39	27.18 (20.81–37.75)	12.13–68.69
Female					
19–39	124	10.59	144.78	25.89 (20.05–34.33)	11.03–64.93
≥ 40	177	9.28	161.05	27.69 (20.87–37.53)	11.00–93.84

[a] The unit is mmol/mol

UOA/UCr ratio ($P < 0.05$, Figure S1). An inspection of UOA/UCr ratios with respect to sex and age were performed. The median (IQR) ratios of UOA/UCr were 24.52 (18.79–31.98) for males and 30.25 (20.90–42.17) for females, which showed a significant difference ($P < 0.001$). Likewise, a positive relationship between age and UOA/UCr ratio were found as displayed in Fig. 1 ($y = 0.3732x + 13.86$, $P < 0.001$). As stratified by sex, Fig. 2 exhibits that the mean UOA/UCr ratio changes across five age groups as observed visually. Furthermore, the Kruskal–Wallis test was applied to check the significance of the UOA/UCr ratio across five age group in each sex. The results showed that there was a significant age-related difference in the UOA/UCr ratio for males ($P = 0.002$) and females ($P = 0.026$). Therefore, the Harris and Boyd's method was utilized to assess the need for RI partition by sex and age.

Due to the skewed distribution of UOA/UCr ratios, they were log-transformed to achieve a normal distribution for RI partition. There is a significant difference in UOA/UCr ratios between males and females ($P < 0.001$) (Table S2), indicating the importance of sex partitioning ($Z = 6.29$, $Z^* = 4.77$, Table 1). After deciding on sex-based partitioning, an assessment of partitioning by age was carried out. Based on the calculated Z and Z^* values (Table 2), three age groups were formed both in males (19 to 29, 30 to 39 and ≥ 40 years) and females (19 to 39 and ≥ 40 years). As a result, The LC assay-specific adult RI for UOA/UCr ratio was determined with stratification by both sex and age as shown in Table 2. A two-sided 95% RI for UOA/UCr ratio was determined to be 8.499–53.39 with a median value of 23.18 mmol/mol for 19–29 years, 10.25–61.29 with a median value of 24.28 mmol/mol for 30–39 years, and 12.13–68.69 with a median value of 27.18 mmol/mol for ≥ 40 years in males, while 11.03–64.93 with a median value of 25.89 mmol/mol for 19–39 years, 11.00–93.84 with a median value of 27.69 mmol/mol for ≥ 40 years.

External quality assessment

To ensure the reliability of our routine clinical test results, we take an active part in PT from CAP. Up to now, enzymatic assays have been used by the majority of laboratories as exhibited in Table 3. Although response was not formally graded due to insufficient peer group data for our LC-based method, but the percentage differences between our method and enzymatic method were within ± 7.26%, indicating reliable and comparable testing results from our laboratory-developed LC method.

Table 3 The PT results of our LC-based method compared with enzymatic method from CAP

	LC (μmol/L)	Enzymatic method (μmol/L)		Percentage difference[a] (%)
		Mean	SD	
202,210				
KSA-01	210.0	200.24	30.23	4.87
KSA-02	1050.0	1037.40	184.86	1.21
KSA-03	90.0	92.90	18.30	−3.12
Lab(*N*)	1	40		
202,302				
KSA-04	1140.0	1083.85	122.66	5.18
KSA-05	80.0	86.26	21.17	−7.26
KSA-06	200.0	187.24	32.45	6.81
Lab(*N*)	1	39		

[a] Percentage difference, calculated as [(LC assay-based results- enzymatic method-based results) / enzymatic method-based results] × 100%

The variables associated with UOA/UCr ratios

Many individuals suffer from different physiological conditions as critical links to diverse spot urinary oxalate levels. As a result, for the investigation of factors affecting UOA/UCr ratios, 381 individuals with full demographic details, including BMI, blood pressure, and renal function indicators such as UALB/UCr, UREA, Cr, UA, and eGFR, were further examined (Table 4). The UOA/UCr ratios ($n = 381$) showed a significant correlation with sex ($r = 0.23$, $P < 0.01$) and age ($r = 0.36$, $P < 0.01$) (Fig. 3), in line with the results in Table S2 and Fig. 2. Data analysis stratified by sex revealed significant differences in OA, UCr, OA/UCr, UALB/UCr, UREA, Cr, UA, eGFR between males and females, all with $P < 0.001$. Males had higher UOA levels than females, but exhibited lower UOA/UCr ratios (Table 4). These findings were consistent with what was observed in the population in Table S2.

A linear regression was conducted between log-transformed UOA/UCr ratios and various associations including sex, age, BMI, SBP, SDP, UALB/UCr, UREA, Cr, UA and eGFR. Table 5 outlines the key predictors of UOA/UCr ratios. In the stepwise forward regression, model 4 featuring the highest adjusted R square of 0.238 included sex, age, UALB/UCr and UREA. In the backward regression, model 7 having the highest adjusted R square of 0.243 incorporated sex, age, UALB/UCr, UREA and eGFR, but VIF of eGFR was 2.110 (above 2.0). Finally, four variables were included to establish the final regression model as following:

Table 4 Baseline characteristics of the reference subjects for studying relevant factors ($N = 381$)[a]

	All	Male	Female	*P*
N (%)[b]	381	200(52.49%)	181(47.51%)	0.330
Age (years)	46.0(34.0–55.0)	46.5(34.0–55.8)	46.0(34.0–55.0)	0.720
BMI (kg/m^2)	23.91 ± 3.90	23.58(20.80–26.32)	24.03 ± 3.64	0.177
SBP (mm Hg)	122(112–134)	126(116–141)	119(108–131)	< 0.001
DBP (mm Hg)	76(70–85)	80(73–88)	72(68–81)	< 0.001
Urine				
UOA (mmol/L)	0.41(0.32–0.53)	0.44(0.35–0.56)	0.38(0.28–0.50)	< 0.001
UCr (mmol/L)	15.45(10.55–21.63)	18.03(13.86–23.14)	12.02(7.724–18.61)	< 0.001
UOA/UCr (mmol/mol)	27.66(20.46–37.37)	24.58(19.17–32.81)	32.48(21.56–41.70)	< 0.001
UALB/UCr (μg/mg)	5.80(4.20–8.95)	5.00(3.50–7.40)	6.70(5.05–10.95)	< 0.001
Plasma				
UREA (mmol/L)	4.84(4.10–5.65)	5.10(4.22–5.87)	4.53(3.89–5.40)	< 0.001
Cr (μmol/L)	70.0(58.0–81.0)	80.0(74.0–88.0)	58.0(53.0–65.0)	< 0.001
UA (μmol/L)	324(261–399)	385 ± 87	268(221–318)	< 0.001
eGFR (ml/min/L)	102.6(92.45–111.8)	100.1(89.10–109.7)	105.2(96.8–115.3)	< 0.001

[a] Normally distributed data are presented as mean ± SD while normally distributed data

[b] Chi-Square test

Table 5 Associations with UOA/UCr ratios

Variable	Estimated regression coefficient				Collinearity statistics	
	Beta	SE	*T* value	*P*	Tolerance	VIF
Constant	1.272	0.053	24.002	< 0.001		
Sex	0.067	0.018	3.656	< 0.001	0.959	1.042
Age (years)	0.006	0.001	8.692	< 0.001	0.917	1.090
UALB/UCr (μg/mg)	0.000308	0.000117	2.623	0.009	0.947	1.056
UREA (mmol/L)	− 0.042	0.007	− 5.844	< 0.001	0.862	1.160

Data are log-transformed for normal distribution

$$Y = 1.272 + 0.067\ \text{Sex} + 0.006\ \text{Age} + 0.000308\ \text{UALB}/\text{UCr} - 0.042\ \text{UREA}.$$

The final model's predictive factors were responsible for 23.8% of the variation in UOA/UCr ratios, and all variables had strong correlations in Spearman's correlation analysis (Fig. 3).

Discussion

Nearly 60–70% of medical decisions are influenced by laboratory testing [17]. As a result, reference intervals are important for interpreting clinical laboratory tests and ensuring patient care. Oxalate serves as an significant indictor for diagnosis of hyperoxaluria. Up to now, many studies have explored the alterations in the UOA/UCr ratio among patients with calcium oxalate stones [18, 19], but only a limited number have examined the RI of UOA/UCr ratio, especially in adults [6, 10, 20]. In Barratt's study, age-specific reference range of UOA/UCr ratio have been established in four subgroups (< 1 yr: 15–260 mmol/mol; 1 to < 5 yr: 11–120 mmol/mol; 5 to 12 yr: 5.9–150 mmol/mol; > 12 yr: 2.1–83 mmol/mol), and a negative relationship between UOA/UCr ratio and age ($r = -0.50$, $P < 0.0001$) has been found [6]. In Hoppe's research, normal values for the UOA/UCr ratio have been reviewed, and five age-specific RI has been established (0–6 months: < 325–360 mmol/mol; 7–24 months: < 132–174 mmol/mol; 2–5 years: < 98–101 mmol/mol; 5–14 years: < 70–82 mmol/mol; > 16 years: < 40 mmol/mol) [20]. In Elgstoen's study, 56 healthy individuals (23 males/33 females, median age 43 years) has been enrolled for establishing RI of UOA/UCr ratio with the upper limit of 65 mmol/mol, and it has found no age dependence[10]. However, the adult RI of UOA/UCr ratio was not discussed based on large sample size, which might be greatly influenced by age. As a result, a detailed investigation into the RI of the UOA/UCr ratio in adults is necessary to aid in accurate clinical decision-making.

In our study, a total of 608 subjects has been enrolled from those who underwent physical examinations for establishing RI of UOA/UCr ratio. In adults, a significant difference in the UOA/UCr ratio between genders was found ($P < 0.001$). The UOA concentration in females (0.39 (0.28–0.48 mmol/L) was lower than that in males (0.44 (0.35–0.56) mmol/L), while a higher UOA/UCr ratio in females (30.25 (20.90–42.17) mmol/mol) compared to males (24.52 (18.79–31.98) mmol/mol) ($P < 0.001$) was observed as a result of the higher UCr excretion in males (Table S2) [10]. This result was consistent with that shown in Hagen's and Elgstoen's study [10, 21]. However, UOA/UCr ratio was not sgnificantly difference between sexes in both Hagen's and Elgstoen's study, which might result from different sample sizes. In addition, a positive correlation ($y = 0.3732x + 13.86$, $P < 0.001$) between age and the UOA/UCr ratio has been shown (Fig. 1), which opposites to the trend in children as reported in both our study and others [6, 11, 20]. However, Yagisawa' s team reported that the ratio of urinary oxalate to creatinine were higher in older than in younger patients with recurrent calcium oxalate stones [18], which was in line with our result. As stratified by sex, an statistical difference associated with age was found in males and females (Fig. 2). Therefore, it is important to consider sex and age-specific RI of the UOA/UCr ratio. In addition, Harris and Boyd partition criteria was applied to justify whether it would be necessary to partition RI according to sex and/or age. The distribution of UOA/UCr ratios was skewed (Figure S1), and thus a normal distribution was achieved by log-transformation for RI partitioning. The study discovered that sex-specific RI for the UOA/UCr ratio is essential (Table 1), aligning with the recommendation from Clifford-Mobley's study [22]. Furthermore, the age-specific RI for theUOA/UCr ratio stratified by sex have been summarized in Table 2. The 97.5th percentile upper reference limits were approximately within the range reported by Barratt's team (> 12 yr: 2.1–83 mmol/mol) [6]. Although they were outside the range shown in Hoppe's study (> 16 years: < 40 mmol/mol), the median (IQR) UOA/UCr ratio of 26.67(20.01–36.95) mmol/mol was in the range reported in Clifford-Mobley's work [22]. In addition, the external quality assessment presented

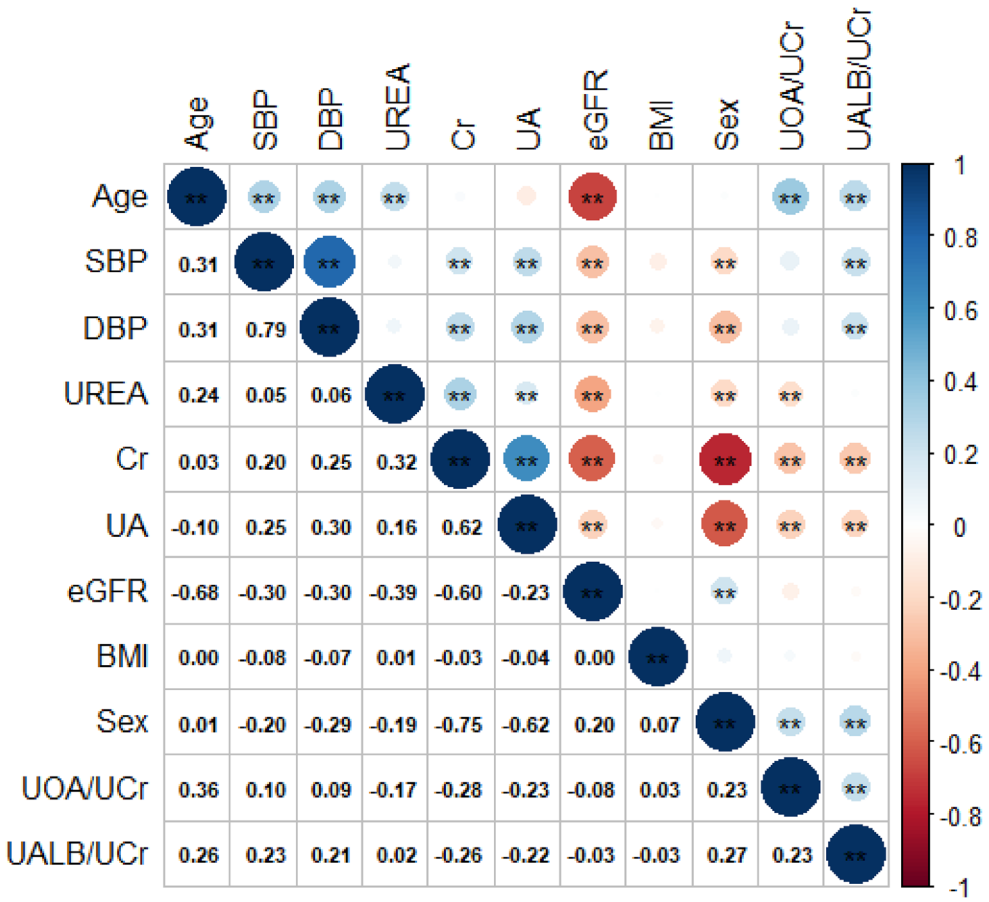

Fig. 3 Spearman's correlation coefficients between parameters in adults ($n=381$). $^{**}P<0.01$, $^{*}P<0.05$. The color bar on the right side represents Spearman's correlation coefficients in the range of − 1.0 (red color) − 1.0 (blue color). The redder the color and the larger the circle, the stronger the negative correlation; the bluer the color and the larger the circle, the stronger the positive correlation

comparable results (percentage differences within − 7.26–6.81%) from this HPLC method and the conventional enzymatic assay, which indicated the reliability of our testing results (Table 3).

Taking into account full demographic details such as BMI, blood pressure and biochemical test, 381 subjects were included for the study on variables linked to UOA/UCr ratios (Table 4). The basic characteristics showed that renal function related biomarkers (UALB/UCr, UREA, Cr, UA and eGFR) exhibited significant difference between males and females, as reported in the literatures [23–27]. The median (IQR) UOA/UCr ratio was 27.66 (20.46–37.37) mmol/mol. The correlation analysis of clinical variables revealed a strong link between sex, age, UALB/UCr, UREA, Cr, UA and the UOA/UCr ratio, but BMI, SBP, DBP, and eGFR did not correlate with the UOA/UCr ratio (Fig. 3). Furthermore, we evaluated a linear regression model to understand the factors affecting the UOA/UCr ratio. The findings showed that age, sex, UALB/UCr and UREA were influential on the UOA/UCr ratio. It is well-known that eGFR is a marker for kidney function estimated from prediction equations based on plasma Cr or cystatin C and demographic variables such as age, sex and race. However, UALB/UCr is a marker for kidney damage, which precedes the decrease eGFR and favorable for early diagnosis [28]. Both UREA and plasma Cr are non-protein nitrogenous waste products, but plasma Cr levels remain normal when renal function is significant impaired [29, 30]. Given that urinary oxalate levels have been proven to positively associated with renal epithelial cell injury [31], thus it might be evident that the UOA/UCr ratio variation is greatly associated with UALB/UCr and UREA. The final multiple linear regression model could be effectively explained using Spearman correlation analysis, revealing significant links bwtween the UOA/UCr ratio and sex ($r=0.23$, $P<0.01$), age ($r=0.36$, $P<0.01$), UALB/UCr ($r=0.23$, $P<0.01$) and UREA ($r=-0.17$, $P<0.01$) (Table 5).

Strengths of this study included the in-depth analysis of RI for UOA/UCr ratios in adults, and the consideration

of variables associated with UOA/UCr ratio for proper interpretation of results in individuals. However, this study's principal limitation was its cross-sectional design, which meant that only the clinical examinations previously requested by the clinician could be evaluated, leaving other factors unevaluated. In addition, the number of samples to establish the RI of the UOA/UCr ratio by sex and age might be insufficient.

Conclusion

This study outlined sex and age-specific RI for urinary oxalate in adults using an HPLC-based method assured by a CAP proficiency test. In addition, we found that the UOA/UCr ratio was affected by UALB/UCr and UREA, and the derived equation could be used to interpretate the individual variability of the UOA/UCr ratio. Considerable differences were found in studies involving different ethnicities and analysis methods. This population and method-specific RI will be useful for diagnosis of hyperoxaluria in adults. However, the need for harmonization and standardization of oxalate assay persists.

Abbreviations

RI	Reference interval
PH	Primary hyperoxaluria
HPLC	High performance liquid chromatography
CAP	College of American Pathologists
UOA/UCr	Urinary oxalate/urinary creatine
BMI	Body mass index
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
UALB	Urinary albumin
UCr	Urinary creatinine
Cr	Plasma creatinine
UREA	Urea
UA	Uric acid
eGFR	Estimated glomerular filtration rate
PT	Proficiency testing
IQR	Interquartile range
VIF	Variance inflation factor

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-025-02657-6.

Supplementary Materials 1

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Author contributions

YS: conceptualization, data curation, investigation, methodology, formal analysis, validation, software, writing-original draft; XL: data curation, investigation, methodology; QG: supervision, visualization. WJL: data curation, visualization. LMC: writing-review and editing, project administration, supervision, visualization. All authors read and approved the final manuscript.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (IRB Approval Number: TJ-IRB20230837).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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