Estimation of 24-hour urine protein excretion using urine albumin-to-creatinine ratio

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Research article

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Abstract

Background There is still a lack of quantitative description of the relationship between urine albumin-to-creatinine ratio (ACR) and 24-hour urine protein excretion (24h UPE). We aimed to study the correlation between 24h UPE and urine ACR and develop a prediction model for 24h UPE employing urine ACR.

Methods This was a retrospective and observational study. All individuals with same-day urine ACR and 24h UPE tests in Sichuan Provincial People’s Hospital from September 1, 2018 to December 31, 2019 were enrolled in the study. Correlation and agreement between urine ACR and 24h UPE were evaluated. A prediction model of 24h UPE was developed and validated.

Results 671 subjects were identified. Urine ACR correlated well with 24h UPE (Pearson's coefficient after natural logarithm transformation = 0.908; p<0.001) and the agreement was consistently good (overall ICC = 0.938; 95% CI: 0.928-0.947; p<0.001). Our multivariable transform model had good performance (R^2 =0.869) and high accuracy (RMSE=0.690) to estimate 24h UPE less than 10 g/day.

Conclusions Urine ACR correlates well with 24h UPE in a general population. Our prediction model is an useful tool for estimating 24h UPE less than 10 g/day, however, 24h UPE is still mandatory in situation when the majority of proteinuria is of tubular origin.

Background

Since proteinuria is not only one of the most important manifestations of kidney diseases, but also an essential measurement in monitoring progress and/or prognosis of various glomerular diseases [1], its quantification constitutes an indispensable part in daily practice of nephrologists. The 24 hours urine protein excretion (24h UPE) is considered the “gold standard” method for quantifying proteinuria, however, this test can be cumbersome for patients and is sometimes at a high risk of inaccuracy due to inadequate collection [2].

Another well-accepted approach to evaluate proteinuria is urine protein-to-creatinine ratio (PCR) and urine albumin-to-creatinine ratio (ACR) in spot morning urine samples[3], whose collection is much simpler compared with 24 hour urine. Although there are a number of studies using urine PCR to predict 24h UPE [2-5] or investigating the correlation between PCR and ACR [6], and studies also demonstrated both ACR and PCR in random urine are correlated well [7, 8], there is still a lack of quantitative description of the relationship between urine ACR and 24h UPE [4]. Meanwhile, debate on the rationality and practicality of replacing 24-hour urine collection with a spot morning urine sample always exists [9, 10].

The routine proteinuria examinations carried out in our hospital include 24h UPE and urine ACR tests. However, the correct procedure of collecting 24 hours urine has been found to be poorly understood by patients and often caused errors in the tested results, especially in patients less well educated. To facilitate our clinical practice, we aimed to investigate the quantitative relationship between 24h UPE and
urine ACR, hoping to develop a prediction model to estimate 24h UPE employing urine ACR and limited information on the request sheet, which might provide us an useful tool for assessing proteinuria.

**Methods**

**Study population**

This was an observational study of all individuals who had proteinuria assessment by urine ACR and 24h UPE on the same day from September 1, 2018 to December 31, 2019 in Sichuan Provincial People's Hospital. No exclusion criteria were applied. All patients who had been prescribed proteinuria examinations were instructed both verbally and by a printed instruction to collect urine specimens according to a hospital-wide standardized program launched on June 1, 2018 as follows: (1) The urine ACR sample was the spot morning mid-stream urine sample; (2) To collect the 24-h urine samples, patients were instructed to empty the bladder in the morning and discard the urine, and from that point onward for 24 h, all urine was to be saved in a clean container. At the end of that 24-h period, the bladder was emptied, and that urine was saved. Once the collection completed, the total amount of the urine was to be measured and patients needed to adequately mix up the urine sample by stirring with a clean stick or gently and repeatedly inverting the container for several times, after that a sample of 3-5 ml urine was to be sent to the laboratory with the total amount marked.

This study was approved by the local ethics committee of Sichuan Provincial People's Hospital (No. 2017.124). The institutional review board waived the need for consent since this was an observational analysis of de-identified data. The study was conducted in compliance with local ethnic specifications and principles of the Helsinki Declaration.

**Measurement of proteinuria**

All urine specimens were handled and tested at our central laboratory, and appropriate measurements of preservation and shipment of urine samples were applied. The Beckman Coulter AU5800 (Beckman Coulter, Brea, CA, USA) performed the protein, albumin and creatinine assays. Urine albumin, creatinine and protein concentrations were quantified using turbidimetric immunoassay, picric acid method and end-point method, respectively. Urine ACR (µg/mg) was calculated as the ratio of urine albumin concentration to urine creatinine concentration. The linear detectable range of urine albumin, urine creatinine and 24h UPE tests were 4~300 mg/mL, 2.4~8840 µmol/L and 0~2000 mg/L, respectively.

**Data collection**

Alongside the results of urine ACR and 24h UPE, we also collected basic information on the request form, including age, sex, source, request department, and primary diagnosis. Source of samples were classified into out-patient and in-patient. Request departments were classified into internal medicine and others, with the latter composed of surgery, pediatrics, gynecology and obstetrics, emergency room and others. Primary diagnosis was classified into routine examination and medical condition-related diagnosis, with
the latter composed of proteinuria with unknown origin, nephrotic syndrome, non-dialysis dependent
chronic kidney disease (CKD), dialysis-dependent CKD, connective tissue disease (CTD), and others.

Statistical analysis

Kolmogorov-Smirnov test was used to test the normality of distribution for all continuous data. Natural
logarithmic transformation was applied to move skewness when needed. Descriptive data are expressed
in terms of the median (range) or mean ± standard deviation for continuous data and number
(percentage) for categorical data. Correlation coefficient between urine ACR and 24h UPE was determined
and interpreted as follows[11]: 0.00–0.29 = negligible, 0.3–0.49 = low, 0.5–0.7 = moderate, 0.7–0.9 =
high, and 0.9–0.99 = very high. Intraclass correlation coefficient (ICC) estimates and their 95% confidence
intervals were calculated based on an absolute-agreement, two-way mixed-effects model [12], to evaluate
the agreement between urine ACR and 24h UPE. ICC ≥ 0.85 was interpreted as a good agreement [13]. We
also investigated the correlation and agreement among different subgroups for sex, source, request
department and primary diagnosis as a sensitivity analysis.

To develop and validate a prediction model of 24h UPE, we followed a four-step procedure. First, the
population was randomly divided at a ratio of 7:3 into a development population and a validation
population. Difference of variables between the development and validation populations were tested
using student t test for continuous variables and chi-square test for categorical variables. Second,
Qualitative evaluation of potential significant factors of 24h UPE in the development population was
performed, including chi-square test for categorical variables and univariable regression for continuous
variables. Third, we developed a multivariable natural logarithm transform prediction model of 24h UPE
by multivariable linear regression using a backward elimination approach to select predictors from the
baseline variables. Fourth, we evaluated the performance of the prediction model in the validation
population. Root of mean square error (RMSE) was used to assess the precision. To assess the accuracy,
we classified the validation population into four subgroups based on measured 24h UPE levels: <0.1
g/day (group 1), 1-5 g/day (group 2), 5-10 g/day (group 3), >10 g/day (group 4), and calculated the
percentage of predictive 24h UPE values that fit in the same range of measured 24h UPE values in
different subgroups. A residual plot was also used to illustrate the accuracy. RMSEs and residual plots for
different subgroups of 24h UPE were investigated as a sensitivity analysis.

Statistical analysis was performed using SPSS software package version 22.0 (IBM SPSS, Chicago,
Illinois), with statistical significance set at P<0.05.

Results

A total of 671 subjects with same-day urine ACR and 24h UPE tests results were identified from the
hospital’s database, including 459 in the development population and 212 in the validation population.
Because the distribution of urine ACR and 24h UPE data are known to be highly skewed as confirmed by
Kolmogorov-Smirnov test, we applied a natural logarithm transformation to move skewness, and the
normally distributed Ln (urine ACR) and Ln (24h UPE) were used for subsequent analyses (see
Supplementary Figures 1 & 2). There was no difference in demographic or basic clinical information between the two populations, supporting the comparability of these two groups (see Table 1).

Correlation and Agreement

Ln (24h UPE) correlated significantly well with Ln (urine ACR) in the overall population and different subgroups (Pearson’s coefficients: 0.908, 0.925, 0.876 in the overall, development and validation population; all p<0.001) (see Table 2), and the agreement was also consistently satisfying (ICCs: 0.938, 0.945, 0.924 in the overall, development and validation population; all p<0.001). Sensitivity analysis further supported the excellent correlation and agreement between Ln (24h UPE) and Ln (urine ACR) in all subgroups.

Prediction model of 24h UPE

Qualitative evaluation revealed potential significant factors for Ln (24h UPE) included source (p<0.001), request department (p=0.068), primary diagnosis (p<0.001) and Ln (urine ACR) (p<0.001) (see Supplementary Table 1). Multivariable linear regression revealed Ln (urine ACR), source, and sex were significant predictors (all p<0.001; see Supplementary Table 2). The resulted equation is as follows:

\[
\text{Ln}(24\text{h UPE(g))} = 0.723\times \text{Ln(urea ACR(μg/mg))}-0.150 \text{(if out-patient)}-0.354\text{(if female)}-4.324 \quad (R^2=0.869)
\]

The equation was then back-transformed and translated into an on-line calculator for the readers’ convenience (scan the QR code in Supplementary Figure 3 or visit: http://redcap.samsph.com/surveys/ and enter the code: H7KX7XXD2W).

The overall RMSE of the prediction model in the validation population was 0.690. Sensitivity analysis showed the model was most accurate for predicting 24h UPE of 1-5 g/day with an accurate rate of 81.3% (see Table 3), followed by accuracy rates of 75.1% and 38.5% for 24h UPE of <1 g/day and 5-10 g/day, respectively. Residual error plots also supported a better performance in 24h UPE of <5 g/day (see Supplementary Figure 4).

Discussion

This study demonstrated good correlation and agreement between urine ACR and 24h UPE in a general population. Our multivariable transform model of 24h UPE had a good performance (R²=0.869) and high accuracy (RMSE=0.690), however, the application of the present model should be limited to predicting 24h UPE less than 10 g/day, preferably less than 5 g/day.

Due to the difficulty in adequately collecting 24-hour urine, especially in patients less well educated and/or with poor compliance, there has long been an effort to use results from spot urine to replace 24h UPE during clinical decision making [14-16]. Our findings of an excellent correlation between urine ACR and 24h UPE in not the overall population but also different subgroups were consistent with previous reports on the correlation between these two variables in primary glomerular disease as well as other
diseases with secondary proteinuria[17-19]. Atkins also reported a strong correlation between urine albumin excretion and total protein excretion in the Australian adult population, particularly among the elderly and patients with comorbidities, but concluded urine albumin measurement should not replace total protein excretion test[20]. Our study not only confirmed the good correlation, but also added to literature a practical calculation tool to estimate total protein excretion. The model benefits from the limited number of predictors, making it very easy and convenient to use in daily clinical practice.

Be that as it may, using urine ACR as a sole indicator for quantification of proteinuria has not reached a consensus[9, 10]. Huang et al reported that spot urine ACR was a simple and convenient indicator of significant proteinuria in women with pre-eclampsia [15], however, Katayev et al reported testing for only urine (micro)albumin can miss up to 40% of females and 30.8% of males with gross proteinuria in a US nationwide laboratory network [21]. The difference might result from the different study populations. A fact that can't be avoided is total protein excretion estimated by albuminuria cannot be used when the majority of protein excreted is not mainly due to the impaired filtration, but to over-excretion or impaired absorption[22]. Estimating 24h UPE by urine ACR is suitable for glomerular urine protein, reflected by our results that the model performed best for 24h UPE less than 5 g/day. Therefore, in situation when the major component of proteinuria is from tubular origin, collecting 24-hour urine is still mandatory.

Our prediction model was promising, with high $R^2$ and low RMSE supporting the accuracy of prediction. However, the present results indicated the model did not work for 24h UPE more than 10 g/day. The reason has been discussed above. It is very interesting that the source of samples, i.e. from in-patients or out-patients, had a negative impact on the prediction. To explore the difference of predicted and measured 24h UPE in these two sources, we found samples from out-patients tended to have more positive residual error (see Supplementary Figure 5), meaning the predicted model was likely to over-estimate for out-patients. This result was consistent with the negative sign in front of “out-patient” in the equation. The same trend existed for the females (see Supplementary Figure 6). We speculated that with more data accumulated, these negative regression coefficients might change to counteract the present trend of overestimation. Further improvement is expected since there is a continuous data collection undergoing. The more input we have, the more accurate the prediction model would be.

There were a few limitations which should be put into context. First, this study was based on a retrospective analysis of datasets from a single center. To be translated to a more general population, these results still need further validation. Second, the prediction model has an inherent limitation that it is not suitable for proteinuria of tubular origin, as mentioned earlier, therefore we need to keep this in mind when explaining the results. Third, since we did not routinely measure body weight in our clinic, we were not able to evaluate the adequacy of urine collection, which was usually assessed by comparing the creatinine from the 24-h urine sample collection with the expected creatinine content [1, 23]. Including samples from under-collection might interference the accuracy in assessing the correlation and agreement between urine ACR and 24h UPE. To overcome this limitation, we provided patients with instructions on collecting urine specimens according to a hospital-wide standardized program, and
enrolled every patient with paired urine ACR and 24h UPE results to reduce possible bias. With more data collected, the performance of prediction model is hopefully to improve.

**Conclusions**

In conclusion, urine ACR correlates well with 24h UPE in a general population. Our prediction model is an useful tool for estimating 24h UPE less than 10 g/day, however, 24h UPE is still mandatory in situation when the majority of proteinuria is of tubular origin.

**Abbreviations**

ACR: albumin-to-creatinine ratio; ICC: intraclass correlation coefficient; RMSE, root of mean square error; 24h UPE , 24-hour urine protein excretion.

**Declarations**

*Ethics approval and consent to participate*

This study was approved by the local ethics committee of Sichuan Provincial People's Hospital (No. 2017.124). The institutional review board waived the need for consent since this was an observational analysis of de-identied data.

*Consent for publication*

Not applicable.

*Availability of data and material*

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

*Competing interests*

All authors declare no conflict of interest. The work was not paid for by, or written for, any commercial entity.

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*Authors' contributions*
XL contributed to data collection and analysis, and drafted the revised the manuscript. YLF contributed to the study design, data analysis and interpretation, and drafted the revised the manuscript. ZYH and HDQ contributed to data analysis and interpretation, and critically reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

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References


**Tables**

Table 1. Baseline characteristics of the study population.
Variable | Overall population (n=671) | Development population (n=459) | Validation population (n=212) | P values* |
--- | --- | --- | --- | --- |
Male, n (%) | 326 (48.6%) | 230 (49.4%) | 96 (46.8%) | 0.558 |
Age (y), mean±SD | 46.4±17.0 | 45.8±17.4 | 47.78±15.96 | 0.161 |
urine ACR (μg/mg), median [range] | 236.9 (0.17-12407.86) | 250.2 (0.17-12407.86) | 199.4 (1.57-9609.59) | 0.636 |
24h UPE (g), median [range] | 0.49 (0.002-32.046) | 0.51 (0.002-32.046) | 0.407 (0.014-27.300) | 0.692 |
Source | | | | |
Out-patient, n(%) | 316 (47.1%) | 212 (45.5%) | 104 (50.7%) | 0.240 |
In-patient, n(%) | 355 (52.9%) | 254 (54.5%) | 101 (49.3%) | |
Request department | | | | |
Internal Medicine, n (%) | 587 (87.5%) | 412 (88.4%) | 175 (85.4%) | 0.311 |
Others, n (%) | 84 (12.5%) | 54 (11.6%) | 30 (14.6%) | |
Primary Diagnosis | | | | |
Medical condition-related, n (%) | 465 (69.3%) | 330 (70.8%) | 135 (65.9%) | 0.204 |
General examination, n (%) | 206 (30.7%) | 136 (29.2%) | 70 (34.1%) | |

* Development population vs. validation population.

Abbreviation: SD, standard deviation; urine ACR, urine albumin/creatinine ratio; 24h UPE, 24 hour urine protein excretion.

Table 2. Correlation and agreement between logarithm transformed 24h UPE and urine ACR.

<table>
<thead>
<tr>
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<th>Correlation</th>
<th>Agreement</th>
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<td></td>
<td>Pearson</td>
<td>P value</td>
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<td>Population</td>
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<tr>
<td>Overall</td>
<td>0.908</td>
<td>&lt;0.001</td>
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<tr>
<td>Development</td>
<td>0.925</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Validation</td>
<td>0.876</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.918</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td>0.908</td>
<td>&lt;0.001</td>
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<td>Source</td>
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<td>Out-patient</td>
<td>0.887</td>
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<tr>
<td>In-patient</td>
<td>0.911</td>
<td>&lt;0.001</td>
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<tr>
<td>Request department</td>
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<tr>
<td>Internal Medicine</td>
<td>0.915</td>
<td>&lt;0.001</td>
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<tr>
<td>Others</td>
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<td>Primary Diagnosis</td>
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<tr>
<td>Medical condition-related</td>
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<tr>
<td>General examination</td>
<td>0.866</td>
<td>&lt;0.001</td>
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</tbody>
</table>

Abbreviation: interclass correlation coefficients; 95% CI, 95% confidence interval.

Table 3. The accuracy of the prediction model for different ranges of 24h UPE in the validation population.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of measured 24h UPE</th>
<th>Numbers of predicted 24h UPE</th>
<th>Accuracy</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 g/day</td>
<td>144</td>
<td>109</td>
<td>75.7%</td>
<td>0.679</td>
</tr>
<tr>
<td>1-5 g/day</td>
<td>48</td>
<td>39</td>
<td>81.3%</td>
<td>0.523</td>
</tr>
<tr>
<td>5-10 g/day</td>
<td>13</td>
<td>5</td>
<td>38.5%</td>
<td>0.876</td>
</tr>
<tr>
<td>&gt;10 g/day</td>
<td>7</td>
<td>0</td>
<td>0%</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviation: RMSE, root of mean square error.

**Figures**

![Figure 1](image)

**Figure 1**

Plot of residual error of the prediction model. Note: Residual error equals to measured Ln24h UPE subtracted from predicted Ln24h UPE. Abbreviations: Ln24h UPE, natural logarithm transformed 24h UPE.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- STROBEchecklist.docx
- SupplementaryMaterials.docx