Estimation of 24 hour urine protein excretion using urine creatinine/albumin ratio

CURRENT STATUS: Under Review

BMC Nephrology  ■ BMC Series

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Prescreen

10.21203/rs.3.rs-26735/v1

Subject Areas

Urology & Nephrology

Keywords
urine creatinine/albumin ratio, 24 hour urine protein excretion, correlation, prediction model
Abstract

Background

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

Methods

This was a retrospectively observational study. All individuals with paired urine ACR and 24 h UPE tested on the same day in Sichuan Provincial People’s Hospital during September 1st, 2018 to December 31st, 2019 were enrolled. Correlation and agreement between urine ACR and 24 h UPE were evaluated. A prediction model of 24 h UPE was further developed and validated.

Results

671 subjects were identified. Urine ACR had a good correlation with 24 h UPE in general population (Spearman’s coefficient = 0.939; p < 0.001) but the agreement between these two measurements was not consistently good (overall ICC = 0.870; 95% CI: 0.849–0.888; p < 0.001). Our multivariable transform model of 24 h UPE had good performance ($R^2 = 0.829$) and validated high accuracy (RMSE = 0.0227, rRSME = 3.1%).

Conclusions

Urine ACR has a good correlation with 24 h UPE in general population but is not a reliable surrogate for 24 h UPE. Our prediction model is a useful tool for estimating 24 h UPE, however, 24 h UPE is still mandatory in situations when accurate quantification of proteinuria is required.

Key messages:

1. Urine ACR has a good correlation with 24h UPE but is not a reliable surrogate for 24h UPE.

2. Our prediction model is helpful to estimate 24h UPE, with good performance ($R^2=0.829$) and validated high accuracy (RMSE=0.0227, rRSME=3.1%); however, 24h UPE is still mandatory in situations when accurate quantification of proteinuria is required.

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 (24 h UPE) is 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 test proteinuria is urine protein/creatinine ratio (PCR) and urine albumin/creatinine ratio (ACR) in spot morning urine sample [3], which has the advantages of being more easily accessible compared with 24 h UPE. Although using urine PCR to predict 24 h UPE is supported by a number of studies [2–4], there is still a lack of quantitative description of the relationship between urine ACR and 24 h UPE [4], and there had still been debate on replacing 24-hour urine collection with a spot morning urine sample for proteinuria evaluation [5, 6].

In our institute, the routine proteinuria examinations in out institution include 24 h UPE and ACR other than PCR tests. Here we aimed to investigate the quantitative relationship between 24 h UPE and urine ACR, hoping to develop a prediction model to estimate 24 h UPE using urine ACR result and limited information on the request sheet, which might provide us a useful tool for clinical practice.
Study population

This was an observational study of all individuals who had proteinuria assessment by urine ACR and 24 h UPE, with both tests done on the same day during September 1st, 2018 to December 31st, 2019 in Sichuan Provincial People’s Hospital. No exclusion criteria was applied. Since June 1st, 2018, all patients who had been prescribed proteinuria examinations in our hospital were instructed both orally and by a printed instruction to collect urine specimens according to a hospital-wide standardized procedure 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 ethic 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 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 24 h 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 24 h 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 general 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 skew-distributed data when needed. Descriptive data are expressed in terms of median (range) or mean ± standard deviation for continuous data and number (percentage) for categorical data. Correlation coefficient between urine ACR and 24 h UPE was determined and interpreted as follows [7]: 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 coefficients (ICC), which describe the associations and agreement among units in the same group [8], were also calculated as the index of agreement between urine ACR and 24 h UPE. ICC ≥ 0.85 was interpreted as good reliability (agreement) [9]. Correlation and agreement were also further investigated among different subgroups for sex, source, request department and primary diagnosis as a sensitivity analysis.

To develop a prediction model of 24 h UPE, we followed a three-step procedure. First, the population was randomly divided at a ratio of 7:3 into a development population and a validation population. Qualitative evaluation of potential significant factors of Ln 24 h UPE in the development population was performed, including unpaired t-test for categorical variables and univariable regression for continuous variables. Second, we developed a multivariable transform prediction model of Ln 24 h UPE by multivariable linear regression using a backward elimination approach to select variables from those that had indicated significance from the first
step. The resulted prediction model was then evaluated in the validation set using a residual error plot, root of mean square error (RMSE), and relative root of mean square error (rRMSE). Third, the final prediction equation was back-transformed into an on-line calculator for convenience.

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 paired urine ACR and 24 h UPE tests results were identified from the hospital’s database, including 466 in the development population and 205 in the validation population. Urine ACR and 24 h UPE data were skew-ed distributed and accorded with normal distribution after natural logarithm transformation (see Supplementary Figs. 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).
Table 1
Baseline characteristics of the study population.

| Variable                                      | Overall population (n = 671) | Development population (n = 466) | Validation population (n = 205) | 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     |
| 24 h 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; 24 h UPE, 24 hour urine protein excretion.

Correlation and Agreement

24 h UPE correlated well with urine ACR in the overall population and different subgroups (Spearman’s coefficient: 0.940, 0.937, 0.945 in the overall, development and validation population; all p < 0.001) (see Table 2), however, the agreement was consistently satisfying (ICC: 0.870, 0.841, 0.921 in the overall, development and validation population; all p < 0.001). Sensitivity analysis supported the excellent correlation between 24 h UPE and urine ACR in all subgroups, while the agreement between these two variables was less than appropriate in female patients, in-patients, request departments other than internal medicine and patients tested for general examination.
Table 2
Correlation and agreement between 24 h UPE and urine ACR.

| Correlation | Agreement |
|-------------|-----------|
| Spearman    | Interpretation | ICC | 95% CI | P value | Interpretation |
| Population  |            |     |       |        |              |
| Overall     | 0.940 | < 0.001 | Very high | 0.870 | 0.849–0.888 | < 0.001 | Good |
| Development | 0.937 | < 0.001 | Very high | 0.841 | 0.809–0.867 | < 0.001 | Poor |
| Validation  | 0.945 | < 0.001 | Very high | 0.921 | 0.896–0.940 | < 0.001 | Good |
| Sex         |         |     |       |        |              |
| Male        | 0.946 | < 0.001 | Very high | 0.902 | 0.878–0.921 | < 0.001 | Good |
| Female      | 0.931 | < 0.001 | Very high | 0.793 | 0.744–0.833 | < 0.001 | Poor |
| Source      |         |     |       |        |              |
| Out-patient | 0.939 | < 0.001 | Very high | 0.957 | 0.943–0.967 | < 0.001 | Good |
| In-patient  | 0.928 | < 0.001 | Very high | 0.847 | 0.812–0.876 | < 0.001 | Poor |
| Request department | |     |       |        |              |
| Internal Medicine | 0.942 | < 0.001 | Very high | 0.875 | 0.853–0.894 | < 0.001 | Good |
| Others      | 0.911 | < 0.001 | Very high | 0.811 | 0.708–0.877 | < 0.001 | Poor |
| Primary Diagnosis | |     |       |        |              |
| Medical condition-related | 0.947 | < 0.001 | Very high | 0.881 | 0.857–0.900 | < 0.001 | Good |
| General examination | 0.894 | < 0.001 | high | 0.784 | 0.716–0.836 | < 0.001 | Poor |

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

**Prediction model of 24 h UPE**

Qualitative evaluation indicated potential significant factors for natural logarithm transformed 24 h UPE (Ln 24 h...
UPE) included source (p < 0.001), request department (p = 0.068), primary diagnosis (p < 0.001) and natural logarithm transformed urine ACR (Ln urine ACR) (p < 0.001) (see supplementary Table). Multivariable linear regression resulted the following equation:

$$\text{Ln } 24 \text{ h UPE (g) } = 0.700 \times \text{Ln}(\text{urine ACR (µg/mg)})-0.163 \times \text{if out-patient}-4.383 \quad (R^2 = 0.829)$$

The equation was then back-transformed into an on-line calculator for the readers’ convenience (scan the QR code in supplementary Fig. 3 or visit: http://redcap.samsph.com/surveys/ and enter the code: H7XKTXXDW).

For validation, we tested the prediction model in the validation population. The plot of residual error was illustrated in Fig. 1. The RMSE was 0.0227, and the rRMSE was 3.1%, supporting the accuracy of prediction.

**Discussion**

The findings of this study indicated that urine ACR had a good correlation with 24 h UPE in general population but the agreement between these two measurements was not consistently good. Our multivariable transform model of 24 h UPE had good performance ($R^2 = 0.829$) and high accuracy (RMSE = 0.0227, rRMSE = 3.1%).

Due to the difficulty in accurately collecting 24 hour urine, especially in patients who are less well educated and/or with poor compliance, there has long been an effort to use results from spot urine to support clinical decision making. Our findings of excellent correlation between urine ACR and 24 h 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 [10, 11]. Be that as it may, using urine ACR as a sole indicator for quantification of proteinuria has not reached a consensus [5-6]. Huang et al reported that spot urine ACR was a simple and convenient indicator of significant proteinuria in women with pre-eclampsia [12], however, its use in the general population has little evidence. Our results also indicated the agreement between urine ACR and 24 h UPE was not consistently good. Therefore, urine ACR is more appropriate to be considered as an assisted measurement instead of a surrogate for 24 h UPE. In situations that accurate quantification of urine protein is required, 24 h UPE is still mandatory.

Our prediction model was promising, with high $R^2$ and low RMSE supporting the accuracy of prediction. One advantage of this prediction model is its limited number of predictors, making it very easy and convenient to use in daily clinical practice. It is very interesting that the source of sample, i.e. from in-patients or out-patients, had negative impact on the prediction. To explore the difference of predicted and measured 24 h UPE in these two sources, we found samples from out-patients tended to have more positive residual error (see Supplementary Fig. 4), meaning the predicted model was likely to over-estimate. This result is consistent with the negative sign in front of “out-patient” variable in the equation. Therefore, we speculated that with more data accumulated, the regression coefficient in front of “out-patient” might increase 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 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 inherently limited power for extreme values. We did not apply any exclusion criteria to the population, in order to improve the prediction model’s performance by including as much data as possible. 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 [13]. Including samples from under-collection might interference the accuracy in assessing the correlation and agreement between urine ACR and 24 h UPE. To overcome this limitation, we provided patients with both oral and written instructions on collecting urine specimens according to a hospital-wide standardized procedure, and enrolled every patient with paired urine ACR and 24 h UPE results to reduce possible bias. Continuous investigation with more incoming data might help to further improve the performance of our prediction model.
Conclusions

In conclusion, urine ACR has a good correlation with 24 h UPE in general population but is not a reliable surrogate for 24 h UPE. Our prediction model is a useful tool for estimating 24 h UPE, however, 24 h UPE is still mandatory in situations when accurate quantification of proteinuria is required.

Abbreviations

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

Declarations

Ethics approval and consent to participate

This study was approved by the local ethic 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.

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.

Funding

FYL is supported in part by the Young Scientists Fund of the National Natural Science Foundation of China (No. 81800613). The funder had no role in any aspect of this work.

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 the manuscript and approved the final version.

Acknowledgements

We sincerely thank Dr. Binhuan Wang for his assistance in data analysis.

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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.

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