Original Article

Association between 24-h Urine Urea Nitrogen and Spot Urine Collection

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Objective: The 24-h urine urea nitrogen (UUN) is the standard measurement for nitrogen balance determination, but may be impractical in certain situations. The authors aimed to estimate the correlation between the urine urea nitrogen to urine creatitine (UUN/UCr) ratio from spot urine samples and 24-h urine urea nitrogen.

Materials and Methods: The cross-sectional study enrolled patients admitted to internal medicine wards, Srinagarind Hospital, Khon Kaen University, Thailand, in 2016. Spot urine samples were taken periodically on the same day for 24-h urine collection.

Results: The respective median 24-h UUN, UUN/UCr for spot urine samples from the morning, before noon, and the last void for 34 patients was 4.75 g, 7.53, 6.60, and 7.48. Significant correlations were found between the 24-h UUN and UUN/UCr ratio from spot urine samples for all collection time frames. The UUN/UCr ratio from the sample before noon had the strongest correlation with 24-h UUN (R^2=0.48). The 24-h UUN might be estimated from spot urine samples taken before noon by the equation: 24-h UUN = 0.78 (UUN/UCr from spot urine) +2.6.

Conclusion: The UUN/UCr ratio of a spot urine sample may be an alternative measurement when a 24-h sample is unavailable.

Keywords: Urine urea nitrogen; Urine urea nitrogen to creatinine ratio; Spot urine sample; Non-critical illness; Protein

J Med Assoc Thai 2021;104(Suppl.4): S25-9
Website: http://www.jmatonline.com

Nitrogen is a primary body component. It is required for both tissue protein synthesis and several nitrogenous compounds involved in various functions. The body nitrogen content should be both quantitatively and qualitatively maintained to ensure normal body functions. An evaluation of nitrogen balance considers the quality of protein in the diet and the various processes involved in amino acid and nitrogen homeostasis, which can vary according to the metabolic condition of each individual[(1)]. The irreversible loss of amino acid nitrogen corresponds to net protein catabolism, and net nitrogen loss should correspond to the irreversible catabolism of the amino acids. Nitrogen loss thus represents an integrated measurement of oxidation/catabolism of all amino acids and, by extension of net protein loss.

The nitrogen balance reflects the whole body's metabolism[(1)]. Nitrogen balance determination from 24-h urine collection has been used to monitor protein status, stress level, and treatment efficacy in patients receiving nutrition support. It can be used to derive estimates of human nitrogen (i.e., protein) requirements. The usual approach is based on the regression of nitrogen balance (i.e., the equilibrium between intake and loss) on intake[(1-5)]. A positive nitrogen balance indicates anabolism, whereas a negative nitrogen balance reflects catabolism[(6,7)].

Calculation of nitrogen balanced is achieved by subtracting nitrogen excretion from protein intake utilizing the following formula:

Nitrogen balance = (protein intake/6.25) - (UUN+4)

Where 6.25 is the conversion factor of protein to nitrogen in grams, UUN is the urine urea nitrogen, and 4 is the correction factor of non-urea nitrogen losses[(8)].

The benefits of knowing the nitrogen balance are well-established, but a significant disadvantage of UUN determinations is the requirement of a 24-h urine collection, which may be inconvenient. Since UUN is the only measured value in this calculation and UUN accounts for most of the body's non-wound nitrogen loss, accurate timing in collecting urine is essential for overall accuracy. 24-h urine collection can be impractical. Spills and leakage often occur through accident oversights and/or procedures. In addition, 24-h collection may be inadequate in certain patients, like those who are bedridden or who experience incontinence. A shortened collection time could reduce these obstacles; however, the accuracy of results using a shortened collection time has not been demonstrated. Graves et al proposed a

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How to cite this article:

Watcharasuwanseree S, Rattanachaiwong S, Hongsprabhas P, Pisprasert V. Association between 24-h Urine Urea Nitrogen and Spot Urine Collection. J Med Assoc Thai 2021;104 (Suppl.4): S25-9.

doi.org/10.35755/jmedassocthai.2021.S04.00041

shorter duration of urine collection[9], and Sorkness illustrated that 12-h urine collection could predict 24-h UUN excretion in patients with trauma with an error of <10%[10]. Candio et al demonstrated that 12-h urine collection, but not the 4-h or 8-h determinations, provided satisfactory estimates of 24-h nitrogen excretion in critically ill adults receiving parenteral nutrition[11]. The urine urea nitrogen to urine creatinine (UUN/UCr) ratio of a properly timed "spot" urine specimen may reflect the average rate of urea excretion for the preceding 24-h[12,13].

Due to the limited evidence of the validity of the spot urine UUN/UCr ratio, we aimed to evaluate the correlation between spot urine UUN/UCr and 24-h UUN.

Materials and Methods

Patients selection criteria

Our cross-sectional study recruited patients admitted in the internal medicine wards at Srinagarind Hospital between April 2016 and November 2016. The inclusion criteria were patients 20 years and over, had stable renal function (i.e., stable estimated glomerular filtration rate (eGFR) by CKD-EPI in the past week), receiving the same total calories or total protein during the past week (enteral or parenteral route). Patients were excluded if they (a) had an indwelling urinary catheter, (b) were receiving renal replacement therapy, (c) had significant drainage from wounds, fistula, or drain tubes, or (d) had liver enzyme >5 times the normal upper limit or acute liver failure. All enrolled patients received comprehensive information about the study and provided written informed consent.

Sample size

Sample size calculations were based on the study's primary objective, which was to evaluate the correlation between 24-h UUN and spot UUN/UCr ratio. A sample size of 34 participants was estimated to have adequate power at 0.8.

Urine collection and analysis

Urine samples were collected in plastic containers. Collection began at 06:00 and ended after the final void at the end of the 24-h period. Ten-milliliter samples were collected to measure spot UUN and spot urine creatinine of the first void of the day, one before noon (during 08:00 to 12:00), and the last before bedtime. The rest of each urine volume was collected and combined with the other samples for the 24-h UUN measurement. An in vitro test was done for the quantitative determination of urea/urea nitrogen, as well as creatinine in human serum, plasma, and urine were done using a Roche/Hitachi Cobas C systems (Roche Diagnostic, Indianapolis, USA).

Statistical analysis

The results of the spot UUN and spot urine creatinine first void of the day, before noon (08:00 to 12:00), and the last void before bedtime were not normally distributed, so these were presented as medians and interquartile ranges (IQR). The categorical data were presented as numbers and percentages. The correlation between 24-h UUN and spot UUN were presented as the Pearson's correlation coefficient and the non-parametric Spearman's rank correlation coefficient. The 24-h UUN and each spot UUN/UCr were analyzed for correlations using linear regression equations. The data analyses were performed using STATA version 10.1 (StataCorp, College Station, TX, USA).

The Ethics Committee for the Faculty of Medicine, Khon Kaen University, reviewed and approved the study as per the Helsinki Declaration and Good Clinical Practice Guidelines (Number HE581483).

Results

Thirty-four patients were enrolled in the study. Baseline characteristics are presented in Table 1. The majority of participants were male (55.8%) with a body mass index

Table 1. Demographic and clinical characteristics of patients at baseline

Characteristic	n=34
Sex: male – No. (%)	19 (55.8)
Age – years, median (IQR)	46.5 (31 to 63)
Weight – kg, median (IQR)	54.2 (46 to 67)
Body mass index – kg/m^2, median (IQR)	21.3 (18 to 25.1)
Serum creatinine – mg/dl, median (IQR)	0.7 (0.6 to 0.9)
Estimated glomerular filtration rate – ml/min/1.73 m^2, median (IQR)	106 (77 to 126.3)
Steroid use - No. (%)	11 (32.4)
Principal diagnosis - No. (%)	
Infection	17 (52.9)
Malignancy	8 (23.5)
Obstructive sleep apnea	3 (8.8)
Benign hematologic problem	2 (5.9)
Cardiovascular disease	2 (5.9)
Autoimmune disease	2 (5.9)
Underlying diseases – No. (%)	
Diabetes mellitus	9 (26.5)
Hypertension	8 (23.5)
Cardiovascular disease	3 (8.8)
Chronic kidney disease	8 (23.5)
Stage 1	0 (0)
Stage 2	5 (14.7)
Stage 3	3 (8.8)
Stage 4	0 (0)
Stage 5	0 (0)
Route of nutritional support - No. (%)	
Oral diet/enteral nutrition	33 (97.1)
Parenteral nutrition	1 (2.9)

(BMI) in the normal range for the Thai population. The majority of participants (52.92%) had principal diagnosis of infectious problems. Most were on an oral diet and/or receiving enteral nutrition.

The median and interquartile range for the UUN/UCr ratio of a single urine specimen as well as UUN excretion over the previous 24-h period are presented in Table 2. The correlations between 24-h UUN and spot urine were strong for all time frames. Linear regression equations were calculated for spot UUN/UCr as the 24-h UUN indicated that the urine from before noon provided the highest R^2 correlation (Table 4). The 24-h UUN might be estimated from the spot urine sample taken before noon by the equation from the linear regression analysis: "24-h UUN = 0.78 (UUN/UCr from spot urine) + 2.6". There was no significant reduction in accuracy due to variations in age, sex, BMI, or eGFR (Table 3). Figure 1 shows the scatter plot for the 24-h UUN and UUN before noon (08:00 to 12:00) urine sample, which demonstrated the strongest correlation among the 3 time frames for spot urine sample collection.

Discussion

Nitrogen balance is widely used to estimate total nitrogen body content and protein losses and determine the adequacy of protein intake in patients. Typically, nitrogen balance is calculated using measured 24-h UUN, with various corrections for non-urea urine losses and losses through the skin and feces. The practicality of 24-h urine urea collection can, however, be difficult with various errors, including incomplete collection, bacterial growth, incorrect timing, and incomplete bladder emptying. Shortening collection times could reduce these potential problems; however, the accuracy of shortened collections has not been conclusively demonstrated[9].

The current study enrolled patients from the Internal Medicine ward. The average 24-h UUN value of 4.5 g indicated that the patients were not critically ill or in a catabolic condition. The UUN value was comparable with the previous study conducted among patients with a solid malignancy[14].

Our study revealed that the urine creatinine (UCr) from spot urine samples was relatively consistent, while the spot UUN slightly varied. Therefore, the correlation between the UUN/UCr ratio and 24-h UUN was significant, but not strong. Richard et al investigated the association between the UUN/UCr ratio and 24-h UUN and found diurnal variations in nitrogen and creatinine excretion and the nitrogen/creatinine ratio[14]. The study of UUN excretion rate in people ingesting three normal meals daily at customary meal times showed that UUN excretion was not constant. Spot urea excretion was lowest in the morning just before breakfast and increased shortly after the last meal of the day. Spot urea excretion began falling and declining towards the nadir values observed in the morning, before breakfast[15]. The UUN/UCr ratio had less variation with respect to time of day, indicating a possible advantage in the use of a ratio for predicting 24-h UUN. Middendorf, et al verified the reliability of the UUN excretion rate estimation based on the spot urine UUN/UCr ratio of the last void sample[16]. Ford, et al observed hourly variations in urinary nitrogen loss, which limits the use of abbreviated urine sample determination[17].

Previous studies used spot urine UUN/UCr ratio for certain purposes, particularly as an indicator for dietary intake in patients with chronic kidney disease[18,19]. However, some research suggested that spot urine UUN/UCr ratio were acceptable to predict protein intake at group level rather to at individual level[20]. According to our data, the UUN/UCr ratio from a single voided spot urine sample at any time-i.e., the first void of the day, before noon, and the last void-was

Table 3. Association between age, BMI, eGFR, and 24-h urine urea nitrogen

Factor	Pearson correlation	p-value
Sex	0.013	0.94
Age	0.128	0.47
BMI	-0.082	0.64
Serum creatinine	0.038	0.83
eGFR	-0.120	0.50

BMI = body mass index; eGFR = estimated glomerular filtration rate

Table 2. Median and interquartile range of urea and creatinine values from urine specimens

	24-h urine	First void spot urine	Before noon spot urine	Last void spot urine
UUN: median (IQR)	4.75 g (3.80 to 7.00)	421 mg/dl (253 to 699)	380 mg/dl (197 to 609)	425 mg/dl (178 to 680)
UCr: median (IQR)	0.7 g (0.5 to 0.9)	59.9 mg/dl (31.8 to 91.0)	54.0 mg/dl (22.1 to 99.7)	57.2 mg/dl (22.6 to 83.8)
UUN/UCr ratio: median (IQR)	7.68 (5.18 to 10.00)	7.53 (4.49 to 9.31)	6.60 (4.74 to 9.56)	7.48 (5.78 to 11.34)

UUN = urine urea nitrogen; UCr = urine creatinine; UUN/UCr = urine urea nitrogen to urine creatinine ratio; IQR = interquartile range

Table 4. Prediction of 24-h urine urea nitrogen (Y) from nitrogen creatinine ratio (X)

Collection time	Regression equation	Correlation coefficient (R^2)	p-value
First void of the day	Y=0.91, X+2.32	0.47	<0.001
Before noon urine	Y=0.78, X+2.6	0.48	<0.001
Last void	Y=0.92, X+3.38	0.33	<0.001

significantly correlated to the 24-h UUN. Morning samples—from the first void and the sample before noon—provided a higher correlation coefficient than the last void sample. The most accurate correlation was from the spot urine sample obtained before noon. The regression equation yielded "Y = 0.78X + 2.6"—Y is the 24-h UUN and X the UUN/UCr ratio (before noon spot urine sample). The equation has a correlation coefficient of 0.48 with a significant p-value. The equation was not significantly changed after adjusting for potential confounding factors (i.e., renal function, age, sex, or BMI).

Limitations of the study include exclusion of certain populations who have unstable urine urea and creatinine excretions (i.e., critically ill and acute kidney injury patients). We assumed that all of the patients in the study had stable dietary consumption in the 4 days before urine collection.

In conclusion, a 24-h urine collection to calculate nitrogen balance should continue to be routinely employed as the gold standard. A spot UUN/UCr ratio of a single voided urine may be used under certain circumstances (i.e., patients with poor cooperation in 24-h urine collection). In such situations, it should be suggested that spot urine collection in the morning, particularly between 08:00 to 12:00 may yield the best estimation.

What is already known on this topic?

A 24-h UUN is the gold standard for nitrogen balance determination.

What this study adds?

Spot urine samples may be used as an alternative measurement for monitoring protein metabolism in certain patients and under certain conditions. UUN/UCr from spot urine sample obtained in the morning, particularly between 08:00 and 12:00 correlates with 24-h UUN.

Acknowledgements

The authors thank (a) the Department of Medicine, Faculty of Medicine, Khon Kaen University for publication support, and (b) Mr. Bryan Roderick Hamman under the aegis of the Publication Clinic KKU for assistance with the English-language presentation.

Potential conflicts of interest

The authors declare no conflicts of interest.

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