Validation of the Spot Urine in Evaluating 24-Hour Sodium Excretion in Chinese Hypertension Patients

Weizhong Han, Ningling Sun, Yuanyuan Chen, Hongyi Wang, Yang Xi, and Zhiyi Ma

BACKGROUND

The spot urine method as an alternative approach in estimating daily urine sodium excretion has been proposed for many years. Kawasaki has created an equation to predict daily urinary sodium excretion using second morning urine (SMU) samples which was obtained before breakfast after initial voiding upon arising. Tanaka has developed another equation by examining spot urine samples submitted at random times during the day. A newly published study proposed that the “PM sample,” collected in the late afternoon or early evening before dinner, showed a stronger relationship with actual sodium excretion. We aimed to verify the effectiveness of these methods in evaluating 24-hour urinary sodium in Chinese hypertensive patients.

METHODS

A total of 334 hypertensive participants were eligible to participate in this study. A total of 222 patients provided qualified SMU samples, Post Meridiem (PM) samples, and complete 24-hour urine collections.

RESULTS

Biases using the Kawasaki formula were 2.1 mmol/day for the SMU specimens; for the Tanaka equation, biases of SMU and PM samples were 21.1 and 30.1 mmol/day, respectively. The highest intraclass correlation coefficient (ICC) was 0.64 when the Kawasaki formula was used in PM specimens, with the lowest ICC 0.17 when it is used in SMUs.

CONCLUSIONS

Spot urine method is acceptable for estimating 24-hour urinary sodium excretion in hypertensive individuals. Kawasaki’s formula is useful for estimating population mean levels of sodium excretion from SMU, although it is not suitable for estimating individual sodium excretion.

Keywords: 24-hour collection; blood pressure; estimation; hypertension; spot urine; urinary sodium excretion; validation.

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A chronic high salt intake plays an important role in the onset and aggravation of hypertension, and it is widely recognized that excess salt intake affects the response to antihypertensive drug therapy. Therefore, reducing dietary salt intake is a vital way to prevent cardiovascular diseases and constitutes a potentially important target for the improvement of public health. Daily food consumption recall and 24-hour urine collection are 2 main methods for assessing daily salt intake; usually dietary recall underestimates sodium intake by 30%–50%. Thus, the 24-hour urine collection is considered the gold standard to assess dietary sodium intake. However, it is fairly difficult to get a complete and accurate 24-hour urine collection. Many participants are reluctant because of its cumberliness and inconvenience, and this affects the response rate and practicality of using the test in public health check-up or in population epidemiological surveys.

Based on the above considerations, the spot urine method as an alternative in estimating daily urine sodium excretion attracts increasing attention. Although regarded as less reliable than 24-hour urine collection, it indeed improves the feasibility for this examination. Kawasaki et al. have created an equation to estimate 24-hour urinary sodium levels by measuring the sodium/creatinine ratio (Na/Cr) in the second morning urine (SMU) samples, obtained before breakfast after initial voiding upon arising, with the correlation coefficient 0.782 between estimated and measured 24-hour urine sodium. It is widely applied and validated in some epidemiological population studies. Another method was proposed by Tanaka and his colleagues, who developed an equation to evaluate 24-hour urinary sodium excretion by testing specimens submitted at random times during the day reporting a correlation coefficient 0.54. Using this method, a reasonably good correlation was obtained between predicted and actual 24-hour sodium excretion values. A study published in 2010 noted that the Post Meridiem (PM) sample, collected in the late afternoon or early evening before dinner, had a stronger relationship with the actual 24-hour urinary sodium than the SMU sample. Sodium, potassium, and other electrolytes show a wide variation in urine excretion and hypertensive patients may have a different diurnal pattern of sodium and water excretion than normotensive subjects. The aim of the present study was to explore the validation of Kawasaki and Tanaka formulas in predicting the 24-hour urinary sodium excretion, using SMU and PM samples in Chinese hypertensive patients.

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METHODS

Subjects

From March 2010 to February 2012, 334 hypertensive participants aged 26–76 years living in the Beijing area, who regularly visited Department of Hypertension at Peking University People’s Hospital, were eligible to participate in this study. Written informed consent was obtained from all participants. The following information was recorded: age, height, weight, body mass index, serum sodium (Na) and potassium (K), serum creatinine (Cr), fasting blood glucose, and office blood pressure (BP). Subjects were considered as hypertensive patients if they showed treated or untreated office BP ≥140/90 mm Hg or were normotensive but reported a history of hypertension. A history of hypertension indicates patients with diagnosed hypertension for at least 2 weeks. To avoid the possibility of some specified conditions affecting the urine sodium excretion, for example, the antihypertension medicine especially diuretics, the second hypertension, i.e., primary aldosteronism, and impaired renal function, excluded were subjects taking antihypertension medicine, patients suspected of secondary hypertension, and those with chronic kidney disease (serum creatinine ≥ 133 μmol/L (1.5 mg/dl) or estimated glomerular filtration rate < 60 ml/min/1.73 m²). The study was approved by the ethics committee of Peking University People’s Hospital.

Protocol

Participants who agreed to participate in the study were provided a 4-L screw-capped plastic bottle, a 500 ml plastic beaker, and were instructed to collect a 24-hour urine sample, discarding the first voided urine upon arising in the morning and collecting all voided urine during the subsequent 24 hours, including the first void sample of the following morning. They were also asked to submit SMU and PM samples, respectively. The SMU samples were collected before breakfast after initial voiding on the day the 24-hour urine collection was completed. The PM samples were collected in the late afternoon or early evening before dinner on the day the 24-hour urine collection was initiated. All subjects were required to avoid higher protein diets, strenuous physical activities, which may affect the urine creatinine excretion. Study staff obtained 2 ml aliquots of SMU and PM samples, respectively, with the rest of the urine poured into the 24-hour container. A total of 2 ml aliquots of SMU and PM samples were added to the 24-hour urine collection after analyses. The 24-hour container was then thoroughly mixed and 2 ml aliquots were collected. Participants were also asked to record the time of the first, SMU, PM, and last void on a Collection Sheet. Upon completion of collection, the staff recorded the urine volume in each collection container to determine the total urine volume during the 24-hour collection period. Subjects would be excluded from this study if they do not follow our standard procedure to obtain urine samples.

Urine samples were kept cold with ice packs or in the refrigerator, and eventually frozen at −20 °C. A complete 24-hour urine collection was defined as urine volume ≥500 ml as measured by a technician, recorded collection of ≥20 hours, and reports of spilling urine or missing a void no more than once in 24 hours.16

Measurements

The concentration of each mineral in the 24-hour collection and the urine volume were used to determine the 24-hour excretion amounts. All samples were processed in Laboratory Medicine of Peking University People’s Hospital laboratories using standardized methods. Analysis of sodium and other electrolytes (potassium and chloride) was carried out on a Hitachi DXC800 clinical analyzer.

Physical assessment of patients included weight, height, and systolic and diastolic BP with mercury sphygmomanometers. BP was measured 3 times by trained and certified observers, using standardized mercuric-column sphygmomanometer on the participant in a sitting position after 5 minutes of rest, and the time interval between successive pairs of BP measurements was 2 minutes.

Estimation of 24-hour sodium excretion from spot urine samples

The predicted 24-hour sodium and creatinine excretion were calculated by Kawasaki and Tanaka formulae, separately. The Kawasaki formula8 to estimate 24-hour urinary sodium is given as:

\[
\text{Estimated sodium (mmol/day)} = 16.3 \times \frac{\text{Spot Na (mmol/L)}}{\text{Spot Cr (mmol/L)}} \times \text{Predicted 24-hour urinary Cr (mg/day)}
\]

where Predicted Cr (mg/day) for men = −4.72 × age (years) + 8.58 × weight (kg) + 5.09 × height (cm) − 74.5 and for women = 12.63 × age (years) + 15.12 × weight (kg) + 7.39 × height (cm) − 79.9. The Tanaka formula12 is given as:

\[
\text{Estimated sodium (mmol/day)} = 23 \times (21.98 \times XNa^{0.392})
\]

where \(XNa = \frac{\text{Spot Na (mmol/L)}}{\text{Spot creatinine (mg/dl) \times 10}}\) and Predicted Cr (mg/day) = −2.04 × age (years) + 14.89 × weight (kg) + 16.14 × height (cm) − 2244.45.

Statistical analysis

We first examined the correlation between 24-hour creatinine values predicted by the 2 formulas and the measured amounts, and that between sodium creatinine ratio measured in spot urine and 24-hour collection. The group bias in predicted 24-hour urine excretion was calculated as the difference of predicted and measured for each participant; then, mean of these differences was calculated. Bland–Altman plots were used to evaluate the individual differences between predicted and measured values for
sodium excretion over the distribution of 24-hour sodium excretion, for the Kawasaki and Tanaka equations obtained with the SMU and PM spot urine collections. Paired t-tests were used to assess the statistical significance of differences in predicted vs. measured values. The correlations between estimated sodium excretion from spot urine samples and measured 24-hour urine were quantified by intraclass correlation coefficient (ICC), which is a better index of reliability taking account of the variance between the 2 measures and assessing the degree of consistency of spot urine measurements by 2 methods. The correlations between estimated creatinine values and measured values were quantified by Pearson's correlation coefficients.

The study of Chinese national nutrition and health survey in 2002 reported that in fact the daily average salt intake of the Chinese is 12 g, equivalent of urine sodium 200 mmol/day, which is consistent with the INTERMAP study in 1999, even though the amount of salt intake recommended by China Hypertension Alliance is 6 g, equivalent of urine sodium 100 mmol/day.

On the basis of such diet characteristics, participants were categorized into group I (low, sodium excretion ≤ 100 mmol/day), group II (moderate, sodium excretion > 100 mmol/day and ≤200 mmol/day), group III (high, sodium excretion > 200 mmol/day), based on actual 24-hour sodium excretion. In those 3 subgroups, we reassessed the bias, correlation coefficients between predictive and measured values. Data were presented as mean ± SD. All statistical analyses were conducted by using SPSS (version 19.0, SPSS), except for Bland–Altman plots conducted in SAS version 9.0 (SAS Institute). P < 0.05 was considered statistically significant. All P values were 2-tailed.

RESULTS

Subject characteristics

A total of 222 of 334 hypertensive patients provided qualified SMU and PM samples, and complete 24-hour urine collections validated by the trained staff, whereas 112 patients were excluded from the original cohort mainly because they could not provide urine samples correctly. The profiles of these participants are shown in Table 1. The average 24-hour sodium excretion was 147.9 ± 61.8 mmol/day, corresponding to a salt intake of 9.0 g/day, much higher than the WHO recommended amount of ≤ 5.0 g/day.

Comparison of creatinine excretion and Na/Cr

The predictive 24-hour creatinine values of 1307.5 ± 427.4 and 1359.3 ± 323 mg/day were derived from the Kawasaki and Tanaka formulae, respectively. As shown in Figure 1a,b, the correlation coefficients between measured and predicted 24-hour creatinine values were 0.651 vs. 0.628, with Kawasaki and Tanaka formulae, respectively (both P < 0.001). Figure 1c demonstrated that highly significant r coefficient of 0.905 indicates that creatinine excretion measured by 2 methods was highly correlated (r = 0.905, P < 0.001). As for the Na/Cr, Figure 2a,b demonstrated that the values derived from SMU and PM samples correlated strongly with those measured in 24-hour collection samples (r = 0.507 vs. 0.406, both P < 0.001).

Correlation between estimated and measured sodium excretion

Next, we compared the measured 24-hour urine sodium excretion with the estimated values from SMU and PM samples in Kawasaki and Tanaka formulae, respectively (Table 2). Although mean predicted 24-hour urinary sodium excretion varied by prediction equation and timing of the spot urine collection, the actual mean of sodium excretion (147.9 mmol/day) was more closely characterized by the Kawasaki formula with SMU samples (145.8 mmol/day) compared with the Tanaka formula. Total sodium excreted in measured spot urine collections was low-to-moderately correlated with measured 24-hour sodium excretions in all participants (0.17–0.64) (Table 2). The lowest ICC of 0.17 was obtained with Kawasaki formula used in PM specimens, while the highest of 0.64 was obtained with it in SMU specimens.

Bias between measured and actual sodium excretion

Biases (predicted minus observed 24-hour sodium excretion) with the Kawasaki equation were 2.1 and 84.5 mmol/day for SMU and PM specimens, respectively, indicating a statistically significant difference (P < 0.01); for the Tanaka equation, the bias obtained for PM samples was slightly smaller than that of SMU samples, with no statistically significant difference (21.1 vs. 30.1 mmol/day, P = 0.18).

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Male/female, number</td>
<td>99/123</td>
</tr>
<tr>
<td>Age, years</td>
<td>58.4 ± 14.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165.2 ± 8.4</td>
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<tr>
<td>Weight, kg</td>
<td>70.9 ± 13.4</td>
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<tr>
<td>BMI, kg/m²</td>
<td>25.9 ± 3.5</td>
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<tr>
<td>Office SBP/DBP, mm Hg</td>
<td>143 ± 18/83 ± 12</td>
</tr>
<tr>
<td>sCr, µmol/L</td>
<td>69.0 ± 19.6</td>
</tr>
<tr>
<td>FBG, mmol/L</td>
<td>5.6 ± 1.6</td>
</tr>
<tr>
<td>Serum Na, mmol/L</td>
<td>140.7 ± 2.2</td>
</tr>
<tr>
<td>Serum K, mmol/L</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>24-hour urine Na excretion, mmol/24 hours</td>
<td>147.9 ± 61.8</td>
</tr>
<tr>
<td>24-hour urine Cr excretion, mg/24 hours</td>
<td>1249.2 ± 61.1</td>
</tr>
<tr>
<td>24-hour urine volume, L</td>
<td>1.8 ± 0.7</td>
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</table>

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; K, serum potassium; Na, serum sodium; sCr, serum creatinine; SBP/DBP, systolic/diastolic blood pressure.
Interestingly, Bland–Altman plots showed an overestimation of 24-hour sodium excretion at lower levels and underestimation at higher levels for both formulas (Figure 3a–d), with some outliers at high sodium levels.

Validation of estimated sodium excretion in subgroups

To further investigate the application of both methods, the subjects were divided into 3 subgroups according to their actual 24-hour urine sodium excretion. Table 3 shows the estimated and measured values in each subgroup. In group I, SMU samples showed smaller bias (45.2, 44.7 vs. 83.1, 64.1 mmol/day) and higher ICC (0.33, 0.29 vs. 0.04, 0.11) compared with PM samples, using either Kawasaki or Tanaka equation. In group II, the predicted values using Kawasaki formula with SMU specimens was much closer to the measured sodium excretion (149.2 vs. 146.7 mmol/day). Meanwhile, the ICCs of SMU samples were higher than those of PM samples (0.48, 0.36 vs. 0.01, 0.04). In group III, both formulas underestimated the 24-hour sodium excretion and the correlation coefficient significantly decreased.

DISCUSSION

The purpose of this study was to assess the validity of the above methods in estimating urine sodium excretion from
spot samples and to determine the most appropriate time for collecting spot urine samples. To the best of our knowledge, this is the first study evaluating urine sodium excretion using spot urine specimens in Chinese hypertensive patients.

Table 2. Comparison of measured urine sodium excretion from 2 samples with values estimated by Kawasaki and Tanaka methods

<table>
<thead>
<tr>
<th>Items</th>
<th>Measured 24-hour excretion</th>
<th>Kawasaki method</th>
<th>Tanaka method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SMU</td>
<td>PM</td>
</tr>
<tr>
<td>Mean (±SD) sodium excretion (mmol/day)</td>
<td>147.9±61.8</td>
<td>145.8±38.1</td>
<td>219.5±21.9</td>
</tr>
<tr>
<td>Range of sodium excretion (mmol/day)</td>
<td>43.4–372.3</td>
<td>49.4–347.5</td>
<td>44.8–483.7</td>
</tr>
<tr>
<td>Bias (mmol/day, 95% CI)</td>
<td>Reference</td>
<td>2.1 (0.2–102.3)</td>
<td>84.5±(0.2–213.4)</td>
</tr>
<tr>
<td>Paired t-test P value(^b)</td>
<td>Reference</td>
<td>0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICC (95% CI)</td>
<td>Reference</td>
<td>0.64 (0.53–0.72)</td>
<td>0.17 (0.04–0.29)</td>
</tr>
<tr>
<td>P value</td>
<td>Reference</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
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Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient; SMU, second morning urine.

\(^a\)Significantly greater bias than Kawasaki-estimated SMU excretion.

\(^b\)Compared with the 24-hour urine samples.

Figure 3. Bland–Altman plots presenting measured vs. estimated 24-hour sodium excretion using the Kawasaki equation with SMU (a) and PM (b) and Tanaka formula with SMU (c) and PM (d) samples. The upper and lower limits of agreement are equal to the mean difference ± 1.96 × SD of the difference. Abbreviations: SMU, second morning urine.

The hypothesis that 24-hour urinary sodium excretion can be estimated from spot urine was established based on a good correlation of sodium creatinine ratio between 24-hour urine and spot urine.\(^\text{24}\) Although Kawasaki and Tanaka
formulas differ in creatinine estimation, the correlation between the predicted and measured creatinine excretion levels was high for both \( r = 0.651 \) and \( 0.682 \), respectively. For SMU and PM samples, a similar relationship of the sodium creatinine ratio between 24-hour urine and spot urine samples was observed \( (r = 0.507, 0.406) \), indicating that SMU and PM samples are acceptable for estimating 24-hour urine sodium. It is worth noting that several researches ignored the validation of creatinine and sodium creatinine ratio before applying these 2 formulas, which may result in improper use in the calculation of sodium excretion.25–28

In the present study, estimates of sodium excretion from SMU using the Kawasaki formula showed the least bias and most agreement with measured 24-hour excretion. These results are similar to those reported previously in healthy and hypertensive populations.20,21,28 In 2014, Mente et al. in a study including 1,083 individuals aged 35–70 years from 11 countries demonstrated that Kawasaki formula was the most accurate method with less bias (313 mg/day, equivalent to 8.8 mmol/day) and good correlation (ICC = 0.71) among other formulas.20 To our surprise, Kawasaki formula using PM samples showed the largest bias (84.5 mmol/day) and the lowest consistency (ICC = 0.17) among all spot urine specimens, indicating that it may not be appropriate for estimation using samples collected at other times of the day. As far as Tanaka formula is concerned, relative stability with bias values ranging from 21.1 to 30.1 mmol/day and ICC from 0.26 to 0.38 was obtained, for either SMU or PM samples, consistent with earlier studies.21,22

It is concluded from the present study that the timing of spot urine sample collection is very crucial, because it largely determines the formula which should work appropriately. Kawasaki equation was initially developed for use with SMU specimens collected after the first void in the morning; thus, samples collected at wrong times possibly lead to selection bias, exemplified by the results derived from Kawasaki formula using PM samples. On the other hand, Tanaka formula was established as a simple method for estimating the population mean of sodium excretion from spot (“casual”) urine specimens.12 The method is used for comparing different groups, populations, and annual trends of a particular group in health education and on other occasions.13,14,15 However, Cogswell et al.26 reported that specimens collected in the afternoon and evening were more appropriate for assessing sodium excretion compared with morning or overnight samples. However, “morning samples” were defined by these authors as voiding between 8:30 and 12:30, meaning a range

<table>
<thead>
<tr>
<th>Group</th>
<th>SMU sample</th>
<th>PM sample</th>
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<tr>
<td></td>
<td>Kawasaki’s method</td>
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<td>Group I</td>
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<td>Tanaka’s method</td>
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</table>

Abbreviations: CI, confidence interval; ICC, intraclass correlation coefficient; SMU, second morning urine.

*Compared with the 24-hour urine samples (t-test).

### Table 3. Comparison of the estimated and measured urine sodium excretion from 2 samples in the 3 subgroups

<table>
<thead>
<tr>
<th>Group</th>
<th>SMU sample</th>
<th>PM sample</th>
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of collection. As mentioned above, these samples may be not suitable for Kawasaki formula in calculating the sodium excretion. In addition, the subjects involved in that study were young healthy volunteers aged 18–39 years, which is strikingly different from the population evaluated herein.

In subgroup analysis, the bias and correlation between predicted and measured sodium excretion from Kawasaki and Tanaka formulae fluctuated widely (Table 3). Indeed, 2 methods tended to overestimate the actual sodium amount in low salt intake individuals (group I) while underestimating it in the high salt intake population (group III). Fortunately, these 2 methods display a certain stable performance to some extent in moderate salt intake patients (group II, more than half of the participants). These findings further demonstrated the large variation in sodium excretion and the complexity to derive data from single urine specimens. Several reasons can explain this situation. First, the subjects involved in the present study were much different from those in former researches in height, weight, and age, as well as the known differences in these characteristics between the Oriental and Western populations, which perhaps affect the basis and effectiveness of such predictive formulas. Second, diverse dietary habits, especially salt consumption, have a great influence on the application of these formulae. Third, the number of participants in group I (n = 58) and group III (n = 42) are much fewer than in group II (n = 122). Over- and underestimations in group I and III may be caused by low statistical power. Furthermore, given that Kawasaki and Tanaka methods were likely established based on Japanese dietary and urine electrolyte information, the most precise and practical formula should be developed considering population-based data that take into account the sodium intake by Chinese.

There are several limitations in our study. First, since daily salt intake varies greatly among individuals, repeated collection 24-hour or spot urine samples and multiple measurements should greatly improve the degree of accuracy in estimating sodium excretion. However, the 24-hour urine collection method is hampered by concerns of high participation and elevated costs, which affect the response rate and practicality of the test. Second, urine creatinine concentration, the most important reference index in both Kawasaki and Tanaka equations, is a concern that should not be ignored. Although individual’s creatinine excretion is considered to be relatively stable, it actually alters daily due to dietary protein intake and vigorous exercise. Taking this relatively sensitive factor into account, patients were suggested to collect spot urine samples avoiding such activities in the present study. Indeed, it is very hard to control patients during this process. Third, the spot urine samples used with the predictive equations to estimate 24-hour sodium excretion were part of the whole 24-hour urine collection, that is not independent; thus, it is likely that the correlations observed are higher than those expected if the spot specimens had been collected on a different day. A previous research mentioned that the correlations between spot and 24-hour urinary sodium excretion collected on different days was 0.18–0.27. Finally, all study participants involved were hypertensive patients, with no-antihypertensive treatment and normal kidney function. Imai et al. and Kawamura et al. reported that Tanaka and Kawasaki equations work well for estimating sodium excretion from the first morning urine and SMU in chronic kidney disease patients and those taking antihypertensive drugs, respectively. So a large-scale calibration study in the Chinese population with more participants and medicated hypertension, chronic kidney disease, or use of diuretics should help better understand how these factors influence the prediction of sodium excretion.

An important finding of our study is that sodium intake in Chinese hypertensive patients could be estimated by the spot urine method. Even though regarded as less reliable than 24-hour urine collection, this method is deemed convenient, and available alternative in assessing population mean salt intake, especially for surveys with large populations. Even though the accuracy of the spot urine still needs to be validated because of the limitation in the assessment of individual difference, it will be a very profitable and useful exploration for hypertension population which increased dramatically in China. But above all, determining a formula based on our own population salt consumption data, which is more suitable for Chinese population, would play a crucial role in preventing and curing high BP.

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DISCLOSURE

The authors declared no conflict of interest.

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Validation of Spot Urine


