Original Communication

Low correlation between morning spot and 24-hour urine samples for estimating sodium intake in an Iranian population: Isfahan Salt Study

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Abstract: *Introduction:* Although difficult, the 24-hour urine sodium excretion is still considered as the gold standard method to estimate salt intake. The current study aimed to assess the validity of using spot urine samples in comparison with the standard 24-hour urine collection to estimate sodium and potassium intake in healthy Iranian adults. *Methods and subjects:* This cross-sectional study was performed on 1099 healthy Iranians aged 18–69 years. Samples of 24-hour and fasting morning spot urine were collected to measure sodium and potassium excretions. Tanaka's formula was utilized to predict the 24-hour sodium and potassium urinary excretions based on the spot values. *Results:* The difference between measured and estimated sodium excretion values was 4265 mg/day (95% CI: 4106–4424; $P < 0.001$) and 2242 mg/day in case of potassium excretion (95% CI: 2140–2344; $P < 0.001$). There was a weak significant correlation between the 24-hour urine sodium and potassium excretion and the predicted values (intraclass correlations: 0.22 and 0.28, respectively; both $P < 0.001$). *Conclusion:* The weak association between the predicted and measured values of sodium and potassium along with the marked overestimation of daily sodium and potassium excretions based on the spot urine and using Tanaka formula indicates that Tanaka formula is not practical for the prediction of sodium and potassium or salt intake in Iranian adults. Using other spot urine sampling times and/or adopting a formula designed based on the characteristics of the Iranian population may increase the validity of spot urine tests.

Keywords: Validation, spot urine, 24-hour urine, sodium, potassium, salt intake

Introduction

There are several approaches to determine the required and safe intake levels of minerals [1]. Sodium (Na) is an electrolyte mineral whose intake assessment is essential for hypertension management [2]. The urinary system handles much of the daily Na intake within 24 hours (24 h) after consumption [3]. The gold standard for Na intake estimation is 24 h urine collection. Although this method is not largely affected by food habits, cooking methods, and observer variability, non-compliance of patients might limit its applicability [4]. It involves a cumbersome procedure with potential limitations such as difficulty in collecting 24 h urine samples, lack of patient cooperation, incomplete urine collection, and under- or over-estimation of salt intake due to inadequate urine pooling (especially in populations with increased Na excreted in sweat). Moreover, 24 h urine collection is associated with high costs in large population studies [5, 6]. Other methods of Na dietary assessment are food records, 24 h dietary recall, food frequency questionnaires, and some brief instruments. Dietary assessment methods have a number of disadvantages including recall bias, reporting errors, difficulty in estimation of the salt added during cooking and at the table, and inaccurate and incomplete food composition tables [4, 7]. Therefore, a novel method, called spot urine, has been recently used to assess Na excretion [5, 8]. Tanaka et al. introduced a simple method by developing a formula

to estimate 24 h urinary Na (24 hUNa) and 24 h urinary potassium (24 hUK) excretion using spot urine Na or K to creatinine (Cr) excretion ratio [9]. The predicted 24 hUNa excretion was significantly correlated with the measured Na excretion using 24 h urine collection among a population of Japanese individuals [9]. However, population-based studies comparing the spot urine method and 24 h urine collection have yielded contradictory results [10–14]. Since various factors such as ethnicity, salt sensitivity, age, and gender can affect the use of spot urine sampling for Na intake estimation [15], the validity of the spot urine method needs to be evaluated in different populations [16]. Therefore, we tried to examine the validity of morning spot urine method by comparing its results with those of the standard 24 h urine collection to estimate daily urinary Na and K excretion as an indicator for daily salt intake.

Methods and subjects

Design and participants

This cross-sectional study was performed on 1461 healthy Iranian adults (age: 18–69 years) in 2013. The participants were selected using multistage cluster sampling. After informing the households, one eligible person was selected from each household. Individuals were recruited only if they aged between 18 and 69 years. The exclusion criteria were history of diabetes insipidus, renal insufficiency, special dietary regimen or fasting at the day and time of sampling, history of using diuretics and oral contraceptives, menstruation, pregnancy, urine volume less than 500 mL/day, and more than one missed voiding. Men and women who aged < 50 years and had 24 h urine Cr (24 hUCr) levels respectively below 20 and 15 mg/dL per kg body weight were excluded. In subjects who aged $\geq$ 50 years, 24 hUCr less than 10 and 7.5 mg/dL per kg body weight was an exclusion criterion for male and female participants, respectively [17]. Finally, 362 individuals were excluded and 1099 participants (504 males and 595 females) were enrolled. The study was approved by the ethics committee of the Isfahan Cardiovascular Research Institute (ICRI) (a World Health Organization collaborative center) and written informed consents were obtained from all participants.

Data collection

All individuals who agreed to participate and presented to the ICRI underwent clinical examination at the first visit. After obtaining the subjects' medical history, their height, weight, and waist circumference were measured using standard methods. Body mass index (BMI) was then calculated as weight divided by height squared (kg/m^2). A trained operator measured the participants' blood pressure manually by using a mercury sphygmomanometer and according to a standard protocol [18]. The first Korotkoff sound was recorded as systolic blood pressure and the disappearance of the sounds (V phase) was considered as diastolic blood pressure. The participants were asked to sit and relax, and their blood pressure was measured twice on each arm (after five minutes of rest). The mean value was calculated for each arm and the higher value was used in data analyses [19]. Venous blood samples were also taken to measure serum biochemical factors including fasting blood sugar and lipid profile. The subjects were then provided with sterile plastic containers labeled with their names and a special code. They were asked to use the container to collect urine samples from 7 am one day to 7 am the next day. They were instructed to discard the first urine on the first day but collect the first urine on the second day. If a person was unable to deliver the urine samples for any reason, the samples were collected at their home. The overall 24 hUNa was calculated via multiplying Na 0concentration by urine volume (in liters). Furthermore, in order to estimate 24 hUNa and 24 hUK based on Na, K, and Cr concentrations in spot samples, spot urine samples were taken on the morning when participants delivered their 24 h urine samples.

Na, K, and Cr concentrations were measured in both 24 h and spot urine samples. In order to assess the accuracy of 24 h urinary samples, Cr concentration was measured using Jaffe method (Technical SMA 12–60) [20].

Statistical analysis

We adopted the Tanaka's prediction method to estimate 24 hUNA and 24 hUK based on spot urine Na (SUNa) and K (SUK), respectively [9]. The following equations were hence used:

$$\begin{aligned}\text{Predicted Cr (PRCr)(mg/day)} &= -2.04 \times \text{age} + 14.89 \times \text{weight (kg)} + 16.14 \\ &\quad \times \text{height (cm)} - 2244.45 \end{aligned} \quad (1)$$

$$\text{Estimated 24 hUNaV (mEq/day)} = 21.98 \times \text{XNa}^{0.392} \quad (2)$$

$$\text{Estimated 24 hUKV (mEq}^{**}\text{/day)} = 7.59 \times \text{XK}^{0.431} \quad (3)$$

where PRCr = predicted value of 24 hUCr;
SUNa = Na concentration in the spot voiding urine;
SUK = K concentration in the spot voiding urine;

SUCr = Cr concentration in the spot voiding urine; and XNa (or XK) = SUNa (or SK)/SUCr × PRCr [11]. In order to convert the Na and K level in mEq/day tomg/day, we multiply these amounts by 23 for Na and 39 for K.

The results were presented as mean ± standard deviation (SD) for quantitative variables and summarized as absolute frequencies and percentages for categorical variables. Paired t-tests were used to evaluate differences between study variables measured through 24 h urine and spot urine methods. Correlations between measured and predicted quantitative values were examined by intraclass correlation coefficients (ICC). Sensitivity analyses based on gender and age groups (i.e. 19–29, 30–39, 40–49, 50–59 and 60–69 years) were also conducted. All statistical analyses were performed with SPSS for Windows 19.0 (SPSS Inc., Chicago, IL, USA). P values less than 0.05 were considered statistically significant.

Results

The mean age of participants was 38.4 ± 10.3 years (range: 18–69 years). Table 1 shows the baseline characteristics of participants including age, anthropometric, blood pressure, serum urea, Cr, Na, K, albumin, fasting blood sugar and serum lipids based on gender.

According to Table 2, the Na and K values estimated based on spot urine were significantly higher than measured 24 h urine values in both genders (P < 0.001). In total population the mean difference between measured and estimated Na and K excretion values were 4265 mg/day (95% CI: 4106–4424; P < 0.001) and 2242 mg/day (95% CI: 2140–2344; P < 0.001), respectively. These differences were larger in females than in males. There were significant ICCs between measured and predicted values of 24 hUNa (ICC = 0.22; P < 0.001; Figure 1) and 24 hUK (ICC = 0.28; P < 0.001; Figure 2). Moreover, the estimated 24 hUNa and 24 hUK amounts were significantly associated with the measured values in both females (ICC = 0.21; P < 0.001 and ICC = 0.27; P < 0.001, respectively) and males (ICC = 0.16; P = 0.05 and ICC = 0.29; P < 0.001, respectively) (Figures 3 and 4). In addition, the ICCs between measured and estimated levels of Na and K excretions were significant based on age group (Table 3).

Table 1. Baseline characteristics and serum biomarker levels in the participants based on gender.

Characteristics	Total (n = 1099)	Female (n = 595)	Male (n = 504)
Age (yr)	38.4 ± 10.3	38.1 ± 9.59	38.6 ± 11.1
Weight (kg)	73.2 ± 13.4	69.0 ± 12.7	78.1 ± 12.5
Height (cm)	1657 ± 9.75	158 ± 6.33	172 ± 6.75
Body mass index (kg/m^2)	26.7 ± 4.27	27.2 ± 4.48	26.0 ± 3.94
Waist circumference (cm)	91.4 ± 11.2	90.1 ± 11.9	93.1 ± 10.2
Systolic blood pressure (mmHg)	113 ± 12.0	109 ± 10.7	117 ± 12.0
Diastolic blood pressure (mmHg)	71.9 ± 9.7	69.2 ± 8.65	75.1 ± 9.80
Serum urea (mg/dL)	13.0 ± 3.63	11.9 ± 3.17	14.4 ± 3.67
Serum creatinine (mg/dL)	0.95 ± 0.17	0.86 ± 0.11	1.07 ± 0.15
Serum sodium (mEq/L)	139 ± 2.62	139 ± 2.50	140 ± 2.73
Serum potassium (mEq/L)	4.27 ± 0.35	4.28 ± 0.35	4.27 ± 0.36
Serum albumin (g/dL)	4.89 ± 0.40	4.78 ± 0.38	5.02 ± 0.39
Serum fasting blood sugar (mg/dL)	94.49 ± 16.47	92.8 ± 14.3	96.5 ± 18.5
Serum total cholesterol (mg/dL)	188 ± 38.4	188 ± 37.7	188 ± 39.2
Serum HDL (mg/dL)	47.4 ± 11.1	50.2 ± 10.9	44.1 ± 10.5
Serum LDL (mg/dL)	105 ± 27.5	104 ± 26.8	106 ± 28.4
Serum triglyceride (mg/dL)	138 ± 75.4	120 ± 60.6	159 ± 85.3

Table 2. Mean of predicted and measured 24-hour urine sodium and potassium excretions based on gender.

	Measured 24 h urine excretion	Predicted 24 h urine excretion	Difference (95% CI)*	P-value
Female				
Sodium (mg/day)	3520 ± 1472	7820 ± 1984	4301 (4102–4500)	< 0.001
Potassium (mg/day)	1991 ± 1043	4230 ± 1305	2239 (2105–2374)	< 0.001
Male				
Sodium (mg/day)	4323 ± 1651	8545 ± 2260	4222(3976–4467)	< 0.001
Potassium (mg/day)	2226 ± 1070	4471 ± 1394	2245 (2091–2399)	< 0.001
Total				
Sodium (mg/day)	3887 ± 1607	8152 ± 2145	4265 (4106–4424)	< 0.001
Potassium (mg/day)	2099 ± 1062	4341 ± 1352	2242 (2140–2344)	< 0.001

*95% CI: 95% confidence interval.

Discussion

The weak association and high difference between the mean measured and estimated 24 hUNa and 24 hUK excretion values in both genders in the present study suggested the limitation of spot urine in predicting 24 hUNa and 24 hUK excretion. The measurement of 24 hUNa excretion has major clinical goals, i.e. it assesses Na, a good indicator of salt intake and an important factor in the implementing salt reduction program and consequently, management of

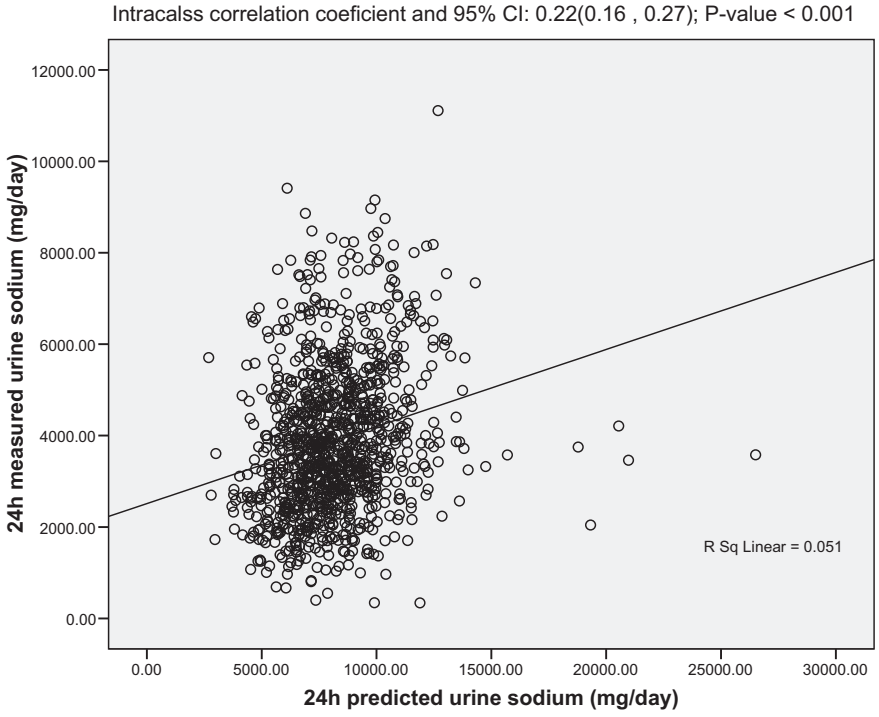

Figure 1. Intraclass correlation coefficient between measured and predicted 24-hour urine sodium excretion in healthy adults population (n = 1099).

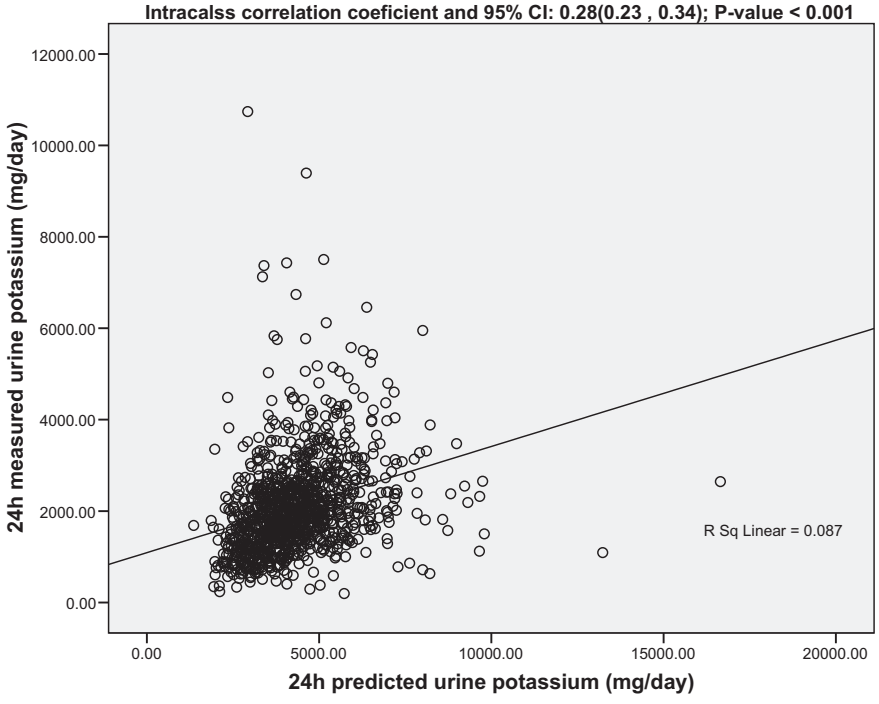

Figure 2. Intra-class correlation coefficient between measured and predicted 24-hour urine potassium excretion on healthy adults population (n = 1099).

hypertension and other diseases [4]. However, Kawasaki et al. concluded that the Na content of a spot urine specimen collected within four hours after the first voiding upon awakening was more suitable for determining the amount of Na intake compared to 24 hUNa collection [11, 12]. In the current study, the 24 hUNa and 24 hUK excretion values

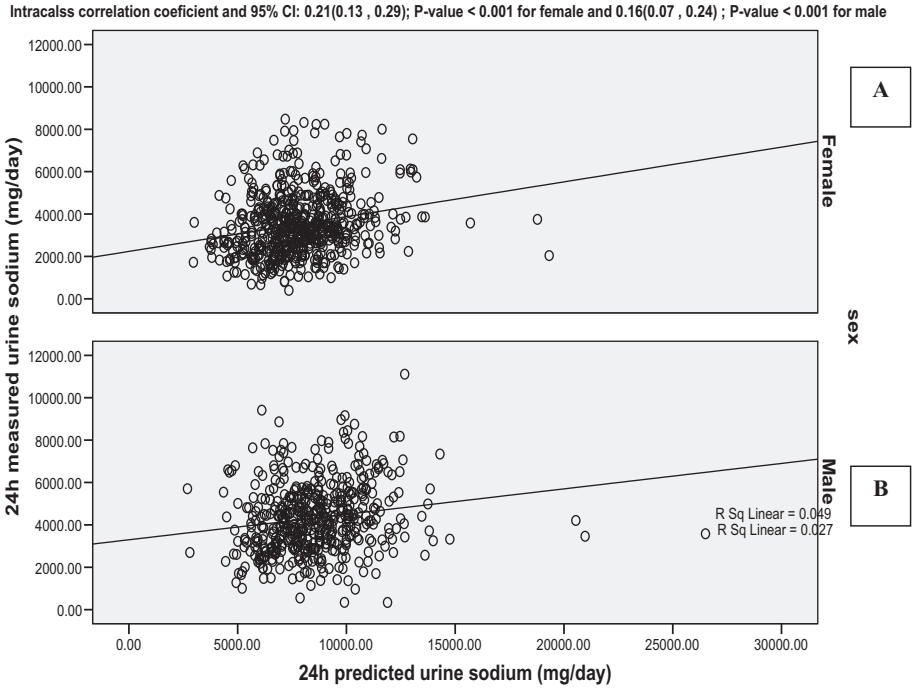

Figure 3. Intra-class correlation coefficient between measured and predicted 24-hour urine sodium excretion in healthy female (A) (n = 595) and male (B) (n = 504).

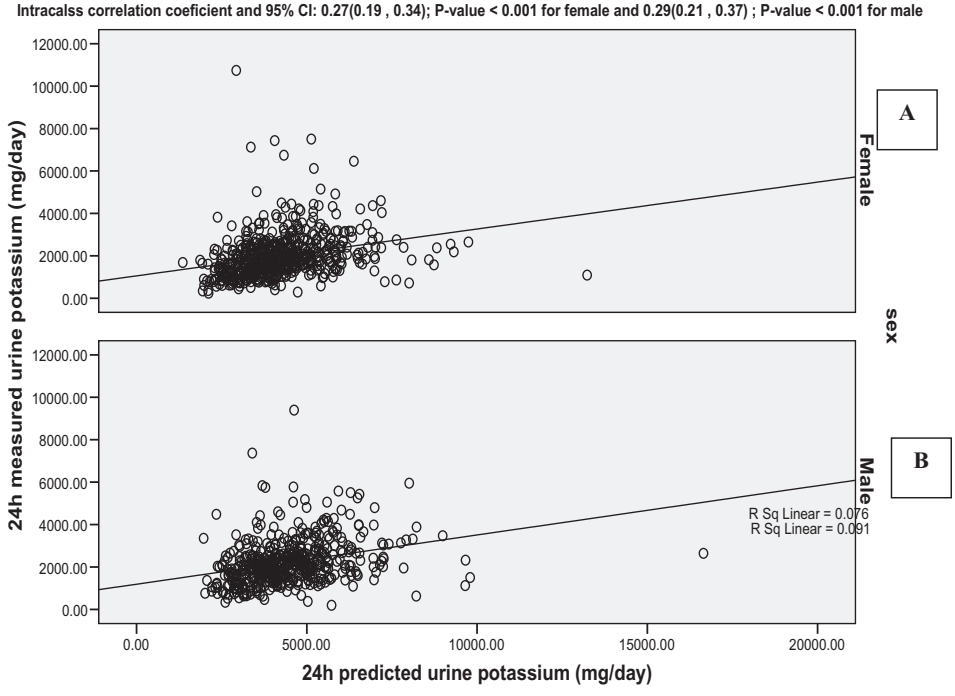

Figure 4. Intra-class correlation coefficient between measured and predicted 24-hour urine potassium excretion in healthy female (A) (n = 595) and male (B) (n = 504).

predicted based on Tanaka's formula were higher than the measured values in both genders. Therefore, salt intake was overestimated based on spot urine. Consistent to our findings, a recent multinational study (in 11 countries) by Mente et al. reported that the mean predicated 24 hUNa was higher than the actual level [13]. Likewise,

Table 3. Intra-class correlation coefficient between measured and predicted 24-hour urine sodium and potassium excretions in healthy adults population based on age group.

Age category (year)	Sodium		Potassium	
	ICC (95% CI)*	P-value	ICC (95% CI)	P-value
19–29	0.32 (0.20–0.43)	< 0.001	0.29 (0.17–0.40)	< 0.001
30–39	0.26 (0.16–0.35)	< 0.001	0.26 (0.16–0.36)	< 0.001
40–49	0.16 (0.05–0.26)	0.002	0.25 (0.15–0.35)	< 0.001
50–59	0.15 (0.08–0.24)	0.003	0.25 (0.09–0.40)	0.001
60–69	0.18 (0.06–0.29)	0.002	0.32 (0.14–0.39)	0.002

*ICC (95% CI): Intra-class correlation coefficient (95% confidence interval).

Swanepoel et al. [21] and Cogswell et al. [16] found predicated 24 hUNa values based on morning spot urine and Tanaka formula to be significantly higher than measured values.

Inconsistent to our findings, Mann et al. [10] suggested the predicted and measured mean 24 hUNa values to be close and in Zhou et al. study 24 hUNa prediction by Tanaka had underestimation [22]. Mann et al. also found that Na excretion predicted from random urine sample in the evening was significantly correlated with actual 24 hUNa [10]. However, no significant correlation was detected between the random urine sample in the morning and the measured value. Hence, Mann et al. concluded that the time of spot urine collection could affect the correlation between predicted and measured 24 hUNa excretion values. They implied that the Na excretion predicted based on spot urine in the midpoint of the 24 h collection period had the strongest correlation with the measured value [10]. We observed a weak significant correlation between measured and predicted 24 hUNa and 24 hUK excretion values. This correlation was stronger in females than in males. Mente et al. showed a higher correlation between predicted and measured 24 hUNa and 24 hUK excretion values among 11 countries [13]. Some other studies have also documented a significant association between predicted and measured 24 hUNa excretion values [23–26]. However, Cogswell reported the correlation between the mentioned values to be stronger in men than in women [16]. Ogura et al. confirmed spot urine sampling as a useful method to estimate Na excretion, especially in patients with lower renal function [23]. Spot urine collection in the morning in the present study might have been responsible for the observed inconsistencies. We found the association between predicted and measured 24 hUNa and 24 hUK values to be weaker in subjects over 40 years. This finding seems rational since Tanaka's formula (utilized in the present study) was developed using a young population [9].

Previous studies have reported a wide range of correlation coefficients between measured and predicted 24 hUNa values. It seems that the administration of a regression equation designed to predict 24 hUNa and 24 hUK excretion in the Japanese population resulted in the overestimation of these values in our study. Therefore, we may need to develop a new formula or modify the existing formula based on the characteristics of the study population.

A major strength of our study was the recruitment of a randomly selected sample which could well represent the Iranian adult population. Furthermore, we did both methods of urinary collection for all enrolled participants and excluded individuals who failed to collect 24 h samples. Since it can be a substantial day to day variability in dietary Na intake (and thus urinary excretion), the main limitation of our study was collecting the 24 h and spot urine samples on different days. However, it was difficult for the subjects to collect both 24 h and spot urine on the same day. Therefore, the spot urine was collected the next morning with a short interval. In addition, Na excretion in the urine does not necessarily imply salt intake as some other sources of Na, e.g. sodium bicarbonate supplements, can affect the measured values.

In conclusion, considering the weak correlation between measured and predicted 24 hUNa and 24 hUK excretion levels, the application of Tanaka's formula, which was originally developed for the prediction of Na excretion in a Japanese population, might have contributed to the lack of congruence between the predicted and measured 24 hUNa and 24 hUK values. Therefore, using this formula, spot urine test is not a practical method for the estimation of 24 hUNa and 24 hUK in the Iranian population. Moreover, the spot urine test overestimated salt intake and was thus not useful in predicting the average 24 hUNa and 24 hUK excretion in our population. Further research is required to modify the formula based on the characteristics of the Iranian population. Such a formula can then be adopted to obtain useful estimates of salt intake and examine whether spot urine samples taken in the afternoon and evening may provide useful estimates of daily Na and K excretions.

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Authors' contributions
AKH, NS, ZA & NM designed research; AB, NM, MG & MJ conducted research; FN analyzed data; and AKH & NM wrote the paper. All authors read and approved the final manuscript.

Conflicts of interests
No conflicts of interest exist.

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