Feasibility of measuring sodium, potassium and creatinine from urine sample dried on filter paper

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Aim: To develop a bioassay for estimation of sodium, potassium and creatinine in dried urine strips and comparing with their respective concentration in liquid urine samples. **Materials & methods:** Urine was collected on filter paper strips, dried at room temperature and, eluted for estimation of sodium, potassium by indirect ion selective electrode method and creatinine by Jaffé method. **Result:** This bioassay was validated based on the US FDA guidelines for bioanalytical method validation and was linear, sensitive, accurate and precise with acceptable recovery and matrix effects. Analytes were stable in dried urine strips during 1 year of storage at 4°C. **Conclusion:** We conclude that the dried urine is suitable for analysis of sodium, potassium and creatinine and offers a convenient alternative for monitoring dietary salt intake.

First draft submitted: 8 November 2018; Accepted for publication: 8 March 2019; Published online: 17 April 2019

Keywords: cardiovascular disease • dried urine strip • electrolytes • hypertension • kidney dysfunction • salt intake

Prevalence of hypertension is on a rise worldwide and is one of the major risk factors for cardiovascular diseases as well as renal failure [1,2]. The high prevalence has been attributed to high dietary salt intake and reduction in population wide salt intake is the key target to bring down number of deaths due to hypertension and cardiovascular diseases [3–5]. Assessing mean population level salt intake is essential for designing salt reduction strategies but is a challenging proposition. Dietary recall and urinary sodium assessment are currently available tools for assessing salt intake. Dietary assessment is labor intensive and also underestimates salt intake. For estimating sodium excretion, 24-h urine is considered as a gold standard but is cumbersome to collect. Assessment of sodium in spot urine is a viable alternative although less robust and valid as compared with 24 h. Several equations have been proposed which predict reasonably well the 24-h sodium excretion from spot urine employing creatinine in the equation to correct for concentrations. Some equations include urinary potassium also to estimate 24-h sodium excretion (INTERSALT). Surveys for assessing population salt intake often employ collection of spot urine in the field and transportation of the collected urine to a central laboratory for assessment of sodium, potassium and creatinine estimation which is logistically difficult. Since bacterial growth is a potential problem with storage of urine, transportation without much delay at lower temperature is advocated to reliably measure sodium, potassium and creatinine. An alternative approach, which has been successfully employed for many analytes in blood, is the use of blood dried on filter paper. Similar to dried blood, urine dried on filter paper can stabilize the analyte, circumventing the need to transport at low temperatures and addition of preservative, which will affect the measurement of analytes. Dried urine was employed for the measurement of creatinine by Balcazar and Meesters [6]. Recent studies describe use of microfluidic paper based analytical device for measurement of creatinine in urine [7–9] and whole blood [10]. Nanoparticle based sensors for sensitive measurement of creatinine directly in urine has been described by Alula *et al.* [11]. Hooshmand *et al.* [12] reported use of a novel sensitive electrochemical nanosensor based on the cadmium selenide quantum dots/ionic liquid mediated hollow fiber-pencil graphite electrode for the measurement of creatinine in urine. To the best of our knowledge, measurement of sodium and potassium has not been demonstrated in dried urine

and in our view would have tremendous application in assessing salt intake, especially in developing countries settings with limited resources and geographical challenges. The objective of the present study was to standardize measurement of sodium, potassium and creatinine in dried urine strip (DUS) and subsequently validation of the DUS measurement in a survey. For the validation of DUS assay, in an ongoing NCD risk factor survey being conducted in India to assess salt intake, we collected urine in both filter and in liquid form. The DUS as well as liquid urine sodium, potassium and creatinine levels were used to estimate the salt intake. We also assessed stability of these analytes after 1 year of storage of dried urine on filter paper at 4°C.

Materials & methods

In the first part of the study, a protocol was established in the laboratory for collection of urine in filter strips, drying them, eluting them and estimating sodium, potassium and creatinine in eluted urine from DUS. Performance of DUS assays were assessed according to US FDA guidelines [13]. For this, urine samples were collected from ten volunteers. In the second part of the study, the assay was validated in DUS samples ($n = 138$) collected in a pilot phase of the National NCDs Monitoring Survey (NNMS) being conducted to assess noncommunicable disease risk factors in India. Sodium, potassium and creatinine were estimated in paired DUS and urine samples collected as part of the survey. In the third part of the study, the long-term storage stability of sodium, potassium and creatinine in DUS stored at 4°C was assessed.

Standardization of DUS assay

Preparation of dried urine strip

To collect the urine sample on filter paper, a rectangular shaped strip (size: 10×2.5 cm) was cut out from 3 MM grade Whatman filter paper sheet (part number: 3030-917) and strip was divided into three parts by length with line marking drawn by a pencil; first part of 2.5 cm was used as a protective cap for handling and identification labeling, second part of 2.5 cm was kept blank for expected urine flow by diffusion and last third part of 5 cm was for urine sample collection (Figure 1).

The filter paper strip was dipped into collected urine sample up to 5 cm line marked on the strip and taken out immediately and allowed to dry completely for 2–3 h in air at ambient temperature (avoiding direct sunlight) by hanging through the protective cap end with the help of sticker holder pin on nonabsorbent thermocol sheet. After drying, filter strips were transferred to a resealable plastic zip bags individually and stored at 4°C until the analysis.

Preparation of calibrators & quality controls on filter paper strips

To eliminate matrix differences, calibrators and controls were also prepared on filter paper similar to the samples. The material used for calibration was ion selective electrode (ISE) high urine standard (reference number AUH1016, Beckman coulter, CA, USA), containing sodium and potassium concentration of 200 and 100 mmol/l, respectively. Multiple calibrators were prepared by serial dilution of this calibrator with deionized water to get five different concentrations for sodium (200, 100, 50, 25 and 0 mmol/l as blank) and five different concentrations for potassium (100, 50, 25, 12.5 and 0 mmol/l as blank). The five calibrator solutions and two quality controls (Liquichek urine chemistry control level 1 [cat no. 397] and level 2 [cat no. 398]; Lot:66760, Bio-rad, CA, USA) were taken on filter paper strip as done for urine samples and dried at room temperature and stored in Ziploc bags at 4°C until analysis. Similar urine based calibrators/controls were prepared on filter paper for creatinine. For creatinine calibration, two levels of quality controls; Liquichek urine chemistry control level 1 (cat no. 397) with target value of 65 mg/dl and level 2 (cat no. 398) with target value of 148 mg/dl (Lot: 66760, Bio-Rad, CA, USA) and a blank consisting of only deionized water (as a zero calibrator) were taken on filter paper strip and were used to plot a calibration curve for estimation of actual creatinine level in dried urine [14]. Randox urine control (reference number – AU2352) with target value 73.5 mg/dl (range 62.6–94.0 mg/dl) taken on filter paper was used as control for creatinine.

Elution of sodium, potassium & creatinine from filter strips

The DUS based calibrators, controls and urine samples prepared as described in the previous sections, were eluted with deionized water. Eight spots of 6 mm diameter each of calibrators, quality controls and subject samples were punched out individually into a 24-well plate (product number: 353047, Becton Dickinson, NJ, USA). Total 350 µl deionized water was added to each well and incubated at 37°C for 30 min with agitation at 120 rpm in an incubator shaker. After incubation, plate was centrifuged at 3000 rpm for 10 min and 200 µl supernatant was taken for analysis.

Figure 1. Diagrammatic representation of dried urine strip bioassay.
DUS: Dried urine strip.

Estimation of analytes in DUS extract

Urinary sodium and potassium were measured in the supernatant obtained from DUS as well as in liquid urine by ISE method on autoanalyzer (AU680 Chemistry analyzer, Beckman Coulter, CA, USA). Urinary creatinine levels were measured in the supernatant and liquid urine by Jaffé method on Roche analyzer (serial number: 2322-03, P800 Modular Analytics, Roche diagnostics, Mannheim, Germany) using commercially available kit (reference 11875418-216, Roche diagnostics, Germany). The reagents for Jaffé reaction consisted of picric acid and sodium hydroxide. A calibration curve was plotted based on the multiple calibrators and values of sodium, potassium and creatinine in controls and urine samples were extrapolated from the calibration curves for respective analytes.

Standardization of DUS bioassay parameters for sodium, potassium & creatinine

The standardization of the DUS assays was performed according to the US FDA guidelines 2001 [13]. The analytical parameters applied were linearity, recovery, LOD, LLOQ, intra-assay CV, interassay CV and storage stability of dried urine on filter paper.

Linearity

The linearity of calibration curve generated from range of calibrators of sodium, potassium and creatinine eluted from filter paper was assessed and considered acceptable if the coefficient of determination (R^2) was ≥ 0.990 .

Recovery

The recovery of sodium, potassium and creatinine from DUS was calculated by comparison of observed concentration of the particular analytes with the actual concentration in urine in liquid form of the particular analytes in a sample dried on filter paper strip using the following equation: recovery (%) = (observed concentration in DUS)/(concentration in urine sample) $\times$ 100. Liquichek urine chemistry control level 1 (cat no. 397) and level 2 (cat no. 398); (lot: 66760, Bio-Rad, CA, USA) were used for recovery of sodium and potassium and for creatinine. Total ten urine samples were dried on filter paper strip and their concentrations were measured in dried and liquid to assess recovery.

Limit of detection & Limit of quantification

Limits of quantification (LOQ) defined as the lowest levels of analytes in the sample, which can be reliably quantified, was arrived by preparing a serial dilution of control for DUS bioassay and analyzing each dilution point in triplicates. Lowest DUS based-control with a CV% of $\leq 20\%$ was defined as LLOQ of the bioassays. The LOD defined as the lowest concentration of analyte that can be detected, not necessarily quantifiable, was calculated using the formula ($\text{LOD} = \text{LLOQ}/3.3$) [6,13].

Precision & accuracy

The precision of the bioassay was determined by the intra and interassay CV% of repeated measures of the bioassay. The average interassay CV was calculated by observed concentrations of five samples repeated in assays on different days and for intra-assay CV, samples repeated in same assay and was mathematically calculated using the standard deviation divided by the overall mean and then multiplied by 100. Accuracy or percentage bias was computed by subtracting observed value in liquid urine from observed value in DUS and dividing this by observed value in liquid urine and multiplying the value obtained by 100.

Validation of DUS assay*Participants*

Participants were drawn from the pilot study of the NNMS, which is being conducted to generate estimates of different NCDs related indicators nationally. Among the participants from the pilot survey, DUS as well as urine in liquid form were collected from 69 participants from Delhi centre (Ballabgarh), representative of north India. From each participant, spot urine as well as 24-h urine was collected making it a total of 138 urine samples. Ethical clearance was obtained from institutional ethical committee for conduct of the study and written informed consent was taken from each participant before the collection of urine sample. Pregnant women, subjects with known history of heart or kidney failure or liver disease, taking therapy with diuretics within the preceding 2 weeks, recent gastrointestinal surgery were excluded from the study.

*Sample collection**24-h urine*

Participants were asked to collect a 24-h urine sample as described in Pan American Health Organization protocols on a day most convenient to them [15]. In the morning of the start of the 24-h period, the participants were told to void the bladder and note the time. 'First-pass urine' was to be discarded. All urine passed thereafter was collected in the container provided, including the first urine of the following morning, with the final time recorded.

Spot urine

Total 30 ml of morning fasting urine sample was also collected from the participant on the next day in a separate sterile sample bottle provided.

Two aliquots each of 24-h and spot urine sample was prepared and transported in ice pack to the Department of Cardiac Biochemistry, AIIMS, New Delhi where aliquots of urine samples were stored at deep freezer (-70°C) till analysis.

Collection of DUS

Total 138 urine samples (morning spot and 24 h of same subject) from 69 participants were also collected on DUS in the field employing the method described above in preparation of DUS. After drying, DUS were transferred to resealable plastic bags individually and transported to the laboratory and stored at 4°C until the analysis. The study was conducted in the months of December 2016 and January 2017 when the ambient temperature was around 15–18°C.

Estimation of analytes in dried & liquid urine

Urinary sodium and potassium were measured by ISE method and urinary creatinine levels were measured by Jaffé method in 138 DUS extracts and liquid urine samples as described previously. The biochemical analysis was completed within a month (February 2017) of sample collection.

Salt intake was calculated from sodium values in 24-h samples by multiplying with total urine volume and 2.5, a factor used to convert sodium (Na) to salt (NaCl) as per WHO guidelines [16]. Since we had pairs of spot and 24-h urine samples, both liquid and DUS, we assessed if performance of DUS will be similar to liquid urine for deriving salt intake from spot urine. INTERSALT equation was applied to derive salt intake in spot urine (Supplementary Table 1)

Bioassay comparison

The urinary sodium, potassium and creatinine values obtained in liquid and in DUS were compared by Pearson-correlations and intraclass correlation coefficient (ICC). The mean difference/bias and agreement between both assays were evaluated using Bland–Altman plots. The percentage deviation/bias was calculated using the equation (dried urine - liquid urine/liquid urine × 100) for each analytes. Average absolute error rate (% error = [absolute deviation from the liquid urine/liquid urine] × 100) was also calculated for each analytes in DUS.

Storage stability

The long-term storage stability of urinary sodium, potassium and creatinine was assessed in DUS after storage time of 1 year (till February 2018) at 4°C. The percentage deviation/bias in the analyte value was computed by subtracting values at baseline (T0) from that at other time periods (Tx) using the following formulae percentage deviation/bias = [(Tx- T0)/T0] × 100.

Statistical analysis

Statistical analysis was carried out using IBM SPSS, version 22.0. The p-values less than 0.01 were considered as significant. To evaluate whether the bias and average absolute error of the DUS method was within acceptable limits, we calculated acceptable total change limit (TCL), which was derived from coefficient of analytical variation (CV₁) and biological variation (CV₂) [17] by using the following formula:

$$\text{TCL} = \sqrt{\{(2.77 \times \text{CV}_1)^2 + (0.5 \times \text{CV}_2)^2\}}$$

Where 2.77 is a factor derived by multiplying 1.96, the CI value for bidirectional changes, with $\sqrt{2}$ because CV₁ is being compared with two results. The CV₁ was obtained from a compilation of QC values over 5 months period for each analyte. Supplementary Table 2 gives CV₁ for sodium, potassium and creatinine obtained by us and used for TCL calculation. A mean percentage deviation greater than 2.77 CV₁ represents a significant difference in analyte concentration. For each analyte, CV₂ was taken from values of biological variations (CV_b) compiled by Ricos *et al.* [18] and the factor 0.5 represents one-half value of the within-subject biological variation (CV₂); a goal set by the College of American Pathologists for imprecision of a method [19,20]. A mean percentage difference of an analyte beyond TCL limits is considered as a significant difference.

Table 1. Analytical parameters of dried urine strips-bioassay.

Characteristics	Sodium (mmol/l)	Potassium (mmol/l)	Creatinine (mg/dl)
Linearity (R^2)	0.997	0.999	0.996
Recovery (%)	99.2	104.4	104.5
LOD	0.95	0.47	0.59
LLOQ	3.12	1.56	2.30
Intra-assay:			
– Precision	4.06	1.52	4.96
– CV%			
Accuracy (bias%)	-0.78	4.47	4.50
Interassay:			
– Precision	5.69	6.27	6.96
– CV%			
Accuracy (bias%)	8.43	8.30	-2.69

Table 2. Observed concentration of sodium, potassium and creatinine.

Analytes	N	Liquid urine at baseline; mean ($\pm$ SD)	Dried urine at baseline			Dried urine at 1 year					TCL (%)
			Mean ($\pm$ SD)	Bias% from baseline liquid urine	Average error rate (%) from baseline liquid urine	Mean ($\pm$ SD)	Bias% from baseline liquid urine	Bias% from baseline dried urine	Average error rate (%) from baseline liquid urine	Average error rate (%) from baseline dried urine	
Sodium (mmol/l)	134	120.98 (63.30)	115.70 (55.46)	-4.36	11.20	116.58 (59.14)	-3.63	0.75	8.9	9.25	$\pm$ 16.43
Potassium (mmol/l)	134	36.70 (20.46)	39.54 (19.08)	7.78	14.97	36.47 (20.44)	-0.63	-8.41	12.13	9.87	$\pm$ 18.19
Creatinine (mg/dl)	133	84.51 (43.86)	89.49 (43.84)	5.89	12.29	77.81 (45.02)	-7.93	-15.01	14.57	14.79	$\pm$ 19.75

SD: Standard deviation; TCL: Total change limit.

Results

Analytical parameters of DUS assay

The analytical performance of DUS assay is depicted in Table 1. The coefficient of determination (R^2) of the calibration curve were >0.990 for sodium, potassium and creatinine, respectively, which indicates an excellent linear relationship between the absorbance and concentration of standards for each analyte. The observed mean recovery was $>95\%$ for sodium, potassium and creatinine, respectively, indicating a satisfactory recovery of analytes from dried urine. The LOD was 0.95 mmol/l, 0.47 mmol/l and 0.59 mg/dl and LLOQ were 3.12 mmol/l, 1.56 mmol/l and 2.30 mg/dl for sodium, potassium and creatinine, respectively. Intra-assay CV was $<5\%$ and the inter-assay CV was $<7.0\%$ for all three analytes. Accuracy of proposed method was assessed as bias percent in within day and day-to-day runs. Within day bias was -0.78, 4.47 and 4.50% and the day-to-day bias was 8.43, 8.30 and -2.69% for sodium, potassium and creatinine, respectively.

Validation of liquid & dried urine bioassay

The results obtained by liquid and DUS assays were compared by linear correlation and Bland–Altman analysis for agreement as shown in Figure 2.

Dried versus liquid urinary sodium levels

The mean estimated urinary sodium levels were 120.98 ± 63.30 mmol/l in liquid samples and 115.70 ± 55.46 mmol/l in dried urine samples. The mean bias was 4.36% and the average absolute error rate was 11.20%, and this value was within the acceptable limit (TCL = $\pm 16.43\%$) (Table 2). The linear correlation between dried and liquid urinary sodium analysis in 134 samples is depicted in Figure 2A. The correlation coefficient (r) was $+0.952$ ($p > 0.0001$). The ICC was 0.934 (95% CI: 0.909–0.953; $p < 0.0001$). Bland–Altman analysis shows good agreement between the dried urine and liquid urine sample for urinary sodium levels (Figure 2B). The

Figure 2. Comparison between dried and liquid urinary sodium, potassium and creatinine analysis in 134 samples: linear correlation and Bland–Altman agreement. For urinary sodium: **(A)** linear regression plots; solid line represents the data best fit ($R^2 = 0.888$); and **(B)** Bland–Altman plot for agreement analysis of dried urine and liquid urine sample. The bias was -5.27 and LoA ranged from -47.56 to 36.99 mmol/l. For urinary potassium: **(C)** linear regression plots; solid line represents the data best fit ($R^2 = 0.906$); and **(D)** Bland–Altman plot for agreement analysis of dried urine and liquid urine sample. The bias was 1.36 mmol/l and LoA ranged from -5.08 to 7.78 mmol/l. For urinary creatinine: **(E)** linear regression plots; solid line represents the data best fit ($R^2 = 0.909$); and **(F)** Bland–Altman plot for agreement analysis of dried urine and liquid urine sample. The bias was 2.65 mg/dl and LoA ranged from -10.35 to 15.65 mg/dl. SD: Standard deviation.

mean difference in urinary sodium levels between both assays was $-5.27 (\pm 21.57)$ mmol/l and the LoA varied from -47.56 to 36.99 mmol/l.

Dried versus liquid urinary potassium levels

The mean ($\pm$ SD) urinary potassium in liquid and dried urine was 36.70 ± 20.46 mmol/l and 39.54 ± 19.08 mmol/l in 134 subjects, respectively, with mean bias of 7.78 %. The average absolute error rate was 14.97% and was within the acceptable limits (TCL-18.19%). Linear regression plot ($R^2 = 0.906$, $y = 0.88 \times +7.34$) of urinary potassium in liquid and dried urine is depicted in Figure 2C and as evident dried urinary potassium is correlated with liquid urinary potassium levels (regression coefficient; $r = +0.953$, $p < 0.0001$). A measure of reliability, ICC coefficient was 0.948 (95% CI: 0.928–0.963; $p < 0.0001$). Figure 2D shows the Bland–Altman plot for agreement analysis of dried urine and liquid urine sample. The mean difference was 1.36 ± 3.28 mmol/l and LoA ranged from -5.08 to 7.78 mmol/l.

Dried versus liquid urinary creatinine levels

The mean (SD) of creatinine values were 84.51 ± 43.86 mg/dl and 89.49 ± 43.84 mg/dl with 5.89% bias in measurement. The average absolute error rate was 12.29% and was within the acceptable TCL (19.75%). Figure 2E shows the scatter plot of urinary creatinine in dried and liquid sample. A strong correlation between both methods (correlation coefficient, $r = 0.953$, $p < 0.0001$) was evident. The ICC was 0.953 (95% CI: 0.935–0.967; $p < 0.0001$). Bland–Altman plot for agreement analysis of dried urine and liquid urine sample is shown in Figure 2F. The mean difference was 2.65 ± 6.63 mg/dl and LoA ranged from -10.35 to 15.65 mg/dl.

Storage stability of analytes in dried urine

Sodium and potassium in dried urine matrix was observed to be biologically stable during storage of 1 year at 4°C when compared with baseline analysis in dried and liquid urine in 134 samples. The mean difference in sodium levels was -3.93 (LoA: -42.52 – 34.66) mmol/l (Figure 3A) and 1.23 (LoA: -24.86 – 27.27) mmol/l (Figure 3B) in dried urinary sodium after 1 year of storage when compared with baseline sodium levels in liquid and dried urine as evident from the Bland–Altman analysis. The mean bias in sodium levels was -3.63 and 0.75% ; and absolute error rate was 8.9 and 9.25% from baseline liquid and dried urine, respectively (Table 2). In Bland–Altman plot for potassium levels, the mean difference was -0.839 mmol/l (LoA: -11.53 – 9.86 mmol/l) (Figure 3C) and -3.74 mmol/l (LoA: -13.51 – 6.03 mmol/l) (Figure 3D) from baseline liquid and dried urine and the bias was -0.63 and -8.41% and absolute error rate was 12.13 and 9.87%, respectively, which was within the acceptable limit (Table 2).

At the end of 1 year of storage, creatinine in dried urine showed large difference and deviation as evident in Bland–Altman plot for agreement analysis (Figure 3E & F). The mean difference in creatinine levels was -6.6 (LoA: -32.78 – 9.58) mg/dl and -11.25 (LoA: -34.47 – 11.97) mg/dl from baseline liquid and dried urine, respectively (Figure 3E & F) and bias was -7.93 and -15.01% and absolute error was 14.57 and 14.79%, which was within acceptable limits (Table 2).

Assessment of salt intake from the sodium, potassium & creatinine values in DUS & liquid urine

Salt intake was calculated from sodium values in 24-h samples by multiplying with total urine volume and 2.5. Since we had pairs of spot and 24-h urine samples, both liquid and DUS, we assessed if performance of DUS will be similar to liquid urine for deriving salt intake from spot urine. INTERSALT equation specific for male and female was applied in 54 spot urine samples for which all required parameters were available (Supplementary Table 3). As evident salt intake assessed using DUS was similar to the values obtained with liquid urine in both 24-h urine samples as well as spot samples. Salt could be reliably estimated in DUS stored for 1 year (Table 3).

Discussion

In the present study feasibility of measuring sodium, potassium and creatinine from urine dried on filter paper strip was assessed. A good correlation was found between values obtained in fresh urine in liquid form and liquid dried on filter paper. The percentage deviation/bias and absolute error were within acceptable limit for all three analytes. Parameters like linearity LLOQ, LOD, intra- and interassay CV for the filter paper assay were within the accepted criteria proposed by US FDA [13]. Good recovery of all the three analytes from filter paper ($>95\%$) suggests suitability of dried urine for the measurement of sodium, potassium and creatinine. Usage of dried urine

Figure 3. Storage stability of sodium, potassium and creatinine in dried urine after 1 year at 4°C and comparison with baseline analysis in dried and liquid urine in 134 samples. For urinary sodium: (A) and (B) Bland–Altman plot for agreement analysis of 1 year dried and liquid urine sample. The mean difference was -3.93 (2SD limits: -42.52–34.66) mmol/l and 1.23 (limits: -24.86 to 27.27) mmol/l in dried urinary sodium after 1 year when compare with baseline sodium levels in liquid and dried urine, respectively. For urinary potassium: (C) and (D) Bland–Altman plot for agreement analysis between 1 year and baseline potassium in liquid as well as dried urine, respectively (mean difference was -0.839 mmol/l [LoA: -11.53–9.86] and -0.374 mmol/l [LoA: -13.51–6.03]). For urinary creatinine: (E) and (F) Bland–Altman plot for agreement analysis. The difference was -6.6 mg/dl (LoA: -32.78–19.58) and -11.25 mg/dl (LoA: -34.47–11.97) mg/dl with baseline dried urine and liquid urine sample. SD: Standard deviation.

Table 3. Validity of 24-h sodium excretion using INTERSALT equation as compared with measured 24-h sodium excretion.

N = 54	Measured 24-h excretion (n = 54)			INTERSALT equation (n = 54)		
	Liquid urine (baseline)	Dried urine (baseline)	Dried urine (1 year)	Liquid urine (baseline)	Dried urine (baseline)	Dried urine (1 year)
Mean (SD) sodium level (mg/day)	2576.9 (1388.7)	2599.1 (1626.1)	2539.7 (1612.8)	3281.5 (736.5)	3232.9 (716.1)	3309.1 (715.8)
Salt intake (g/day)	6.442	6.498	6.349	8.204	8.082	8.2725

SD: Standard deviation.

has been reported for the measurement of iodine [14], creatinine [6,14,21], drugs of abuse [22,23] and HPV testing [24]. To the best of knowledge, measurement of sodium and potassium has not been reported in dried urine on filter strips. A good correlation between levels of sodium, potassium and creatinine urine in liquid form and that in dried urine in strips in the present study suggests that DUS can be reliably employed in community surveys to assess salt intake.

We also found good correlation between creatinine measured in DUS and in liquid urine. Measurement of creatinine from dried urine has been reported in earlier studies. Zava *et al.* [14] estimated urinary creatinine from dried urine on filter paper in 272 healthy individuals by Jaffé reaction in a 96-well plate manually and validated the same with liquid urine bioassay. The author reported a strong correlation between liquid and dried urinary creatinine ($R^2 = 0.9782$) and Bland–Altman analysis showed a mean bias of -3.0 mg/dl and LoA from -22 – 16 mg/dl [14]. In another study, urinary creatinine was determined in 49 paired liquid urine and dried urine samples using a HPLC–DAD assay and a strong association was reported by Passing–Bablok regression, mean difference (bias) of 18.3 mg/dl in Bland–Altman analysis was however high. The author reported that the mean difference was reduced to 0.17 mg/dl for urinary creatinine when regression was adjusted for concentrations with LoA between 20.38 and -20.04 mg/dl in Bland–Altman analysis, [21] but a higher deviation in differences remained constant. A study published by Balcazar and Meesters used the 3 and 5 mm (diameter) dry urine paper disks to determine the urinary creatinine and both paper disks were compared with creatinine values assessed in liquid urine [6]. The bias and LoA observed in our study is comparable with that reported by other authors.

We further found that values of sodium and potassium in DUS stored for 1 year at 4°C was well correlated with values reported in fresh urine sample suggesting long-term stability of DUS for sodium and potassium measurement. The decrease in sodium and potassium concentration was $<15\%$ (bias). However, creatinine was comparatively less stable in dried urine during 1 year of storage with mean difference -6.6 mg/dl (LoA: -32.78 – 19.58) and -11.25 mg/dl (LoA: -34.47 – 11.97) from baseline liquid and dried urine as well as bias was -7.93 and -15.01% , respectively and showed a large deviation than found in baseline measurements in liquid and dried urine (LoA: -10.35 – 15.65 mg/dl); but deviation/bias and absolute error were within the acceptable limit ($\text{TCL} = \pm 19.75\%$). We did not assess storage stability of creatinine in liquid urine. Several studies have reported that urinary creatinine is unaffected by long-term storage [25–27]. Salt intake assessed by DUS sodium, potassium and creatinine values were comparable with that derived from liquid urine, both 24-h and spot urine samples, and could also be reliably measured in DUS stored for 1 year at 4°C .

Limitation of the study was that the urine samples in the present study were collected in a well-controlled environment from Ballabgarh, a field setting close to the laboratory and transported the same day and therefore does not mimic situations involving prolonged storage and delayed transportation. A larger multicentric study using DUS would need to be carried out before its usage in surveys is advocated.

Conclusion

In this study, we have presented a simple bioassay for performing sodium, potassium and creatinine in dried urine on filter paper strip consisting collection of urine in filter strips, drying them, extracting the analytes and estimating sodium, potassium by indirect ISE method and creatinine by Jaffé method using auto analyzer. The LOD was 0.95 mmol/l, 0.47 mmol/l and 0.59 mg/dl and LLOQ were 3.12 mmol/l, 1.56 mmol/l and 2.30 mg/dl for sodium, potassium and creatinine, respectively. Intra-assay and interassay CV was <5 and $<7.0\%$ for all three analytes, respectively. An excellent relationship and agreement was observed between sodium, potassium and creatinine level estimated in dried urine and liquid urine. We conclude that the sodium, potassium and creatinine are highly stable in dried urine and are readily transferable to a liquid phase for analysis and offers a convenient

alternative to collection, transportation and analysis of urine in liquid form. Further confirmation of the study can help to simplify procedures for monitoring population level sodium intake in surveys.

Future perspective

The results obtained in this study confirmed that the analysis of sodium, potassium and creatinine on DUS constitutes a clinically valid alternative method to a liquid phase for analysis and offers a convenient alternative to collection, transportation and analysis of urine in liquid form. Importantly, in this approach dried urine samples can be sent by post to a central laboratory. This method, therefore, provides an excellent opportunity for monitoring dietary salt intake and is likely to simplify the screening of hypertension in the general population and its follow-up of patient especially in developing countries. The perspective is that in few years from now this DUS method will be used for mass screening for preventive action in most countries. A larger multicentric study using DUS would need to be carried out before its usage in surveys is advocated.

Executive summary

Aim

- High prevalence of hypertension has been attributed to high dietary salt intake, which can be monitored by measurement of urinary sodium in 24-h/spot urine in a laboratory.
- The objective of this study was to standardize estimation of sodium, potassium and creatinine in dried urine strips and to validate levels of analytes in liquid urine.

Methods & results

- Urine was collected in filter paper strips, dried at room temperature and, eluted prior to estimation of sodium, potassium by indirect ion selective electrode method and creatinine by Jaffé method.
- Intraclass correlation and Bland–Altman analysis shows good agreement between the dried urine and liquid urine sample.

Conclusion

- We conclude that the sodium, potassium and creatinine are stable in dried urine and are readily transferable to a liquid phase for analysis offering a convenient alternative for monitoring dietary salt intake.

Acknowledgements

Authors would like to thank the participants and team members who took part of this study.

Financial & competing interests disclosure

Authors thank the Indian Council of Medical Research and WHO for providing financial support for National NCDs Monitoring Survey from which this pilot study was carried out. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The institutional review board of All India Institute of Medical Sciences, New Delhi, India approved the study. Informed consent was obtained from the all participants prior to conduct of the study.

Author contributions

M Tarik and R Lakshmy were involved in the design of experiment, data analysis, statistical analysis and the writing of the manuscript. A Ritvik, HR Salve, A Krishnan contributed toward recruitment of the participants for the study and evaluation of manuscript. P Mathur and P Joshi contributed in evaluation of manuscript.

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