Original Research Article

Comparison of urine creatinine values of conventional 24 hr urine and fractional urine

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Abstract
Background: Creatinine clearance is a test used to assess renal function(glomerular filtration rate-GFR)) and also for staging chronic kidney disease (CKD) . Urine creatinine estimation requires collection of 24h urine which is a cumbersome and tedious process. Our study proposes a unique fractional urine collection method for urine creatinine estimation that would entail collection of small volumes of urine each time patient voids over 24h period and then compare this new method with conventional 24hour collection method.
Methods: It is a Cross-sectional, prospective study in tertiary care hospital. Volunteers (57) with normal renal function and chronic kidney disease subjects (22) were recruited. Unique method was compared with a conventional method after centrifugation and with and without preservative. Interclass correlation coefficient (ICC) and Bland Altman analysis was used to evaluate for agreement between the two methods.
Results: All the values were combined without categorization into the subgroups. Creatinine results of fractional urine collection method without preservative (UF1) and with preservative (UF2) was compared to conventional method (U24), ICC was 0.86 (C.I, 0.80 to 0.90) and 0.84 (C.I, 0.77 to 0.89) respectively.
Conclusions: The urine creatinine results of unique collection method is comparable and reliable as traditional 24-hour method.
Keywords: Factional, conventional, urine creatinine

Introduction
Creatinine is an anhydride of creatine or creatine phosphate formed in the muscle and excreted in urine at a constant rate. Concentration of creatinine in 24hr urine is used to estimate the creatinine clearance along with serum creatinine and urine volume, which is an established method to assess the renal function(GFR) and stage Chronic kidney disease. It is also used to calculate completeness of the urine collection, which is further used to calculate 24h excretion of other parameters [1-2]. To avoid the disadvantages with conventional method, our study proposes to collect small fraction of urine, 1 ml , each time patient's voids urine over 24hr period .
Methods
Study type: Cross-sectional, prospective study
Study Place: Tertiary care hospital, Bangalore-Karnataka, India.

Period: October 2016 to September 2017

Selection criteria: Participants included healthy volunteers and CKD patients who attended the Out-Patient clinic belonging to age group between 18 and 60 years, either gender. Patient with history of urinary incontinence or suffering from gastroenteritis and having menstruation at the time of collection were not included in the study. Individuals on urinary catheter or undergoing dialysis, pregnant and lactating women, were also not included in the study. Improper collection or not complying with collection protocol, accidental spillage during collection period or at any time before its measurement for volume/parameter were excluded. We recruited 100 adult subjects for the study.

Procedure: Protocol for 24hurine collection

As per the protocol, urine collection starts in the morning 7am (after discarding first morning void) till 7 am on the next day. Participants were provided with 3 closed containers (one large and two small), a jar, disposable syringes. Large container was meant for 24h urine collection by traditional method with preservative thymol, labeled as U24 h while the two small containers were labeled as UF1 (without preservative) and UF2 (with preservative thymol) for urine collection by proposed new method. Subjects were provided with detailed instructions for collection of urine.

Subjects were instructed to pass urine into the clean jar every time they void. One mL of urine was transferred to UF1 and UF2 each and remaining urine in jar was transferred to U24h container. Jar was cleaned and dried for the next void. This process was repeated till the end of collection period of 24h.

Protocol for urine sampling and analysis

Sample volumes were measured in the three containers (U24h, UF1 and UF2). One aliquot of 1.0 mL each were taken from UF1 and UF2 containers after mixing the samples thoroughly and the same was centrifuged and supernatant was aliquoted. After aliquoting, remaining UF1 and UF2 sample was mixed with U24hr container and from this 1 ml was aliquoted . All urine aliquots (3) were analyzed for creatinine by using modified Jaffe Kinetic method standardized to isotope Dilution mass spectrometry method on Siemens Dimension RxL by Siemens Healthcare Pvt. Ltd. The results were used to compare and evaluate the values collected by the fractional and conventional method.

Ethical approval: Ethical approval was obtained from Institutional ethical clearance.

Statistical analysis: Descriptive statistics was used to describe the demographic data and variables such as urine volume, creatinine concentration in various containers. Bland and Altman Analysis were done to evaluate the extent of agreement between the two methods in different groups. Interclass correlation coefficient (ICC) was used to evaluate samples to assess reliability.

Results

79 subjects were selected for the study, while 21 subjects were excluded from the study since they did not follow the collection protocol. 22 samples in the CKD group were predominantly diabetic nephropathy (n=11) followed by chronic glomerulonephritis (n=5), nephrotic syndrome (n=3). hypertensive nephrosclerosis (n=2) and lupus nephritis (n=1).

Table 1: Demographics of the patient

Particulars	
Mean Age (in years)	43+/- 14
No. of apparently healthy volunteers <0.3mg/dl	57
No. of CKD subjects with protein excretion >0.3mg/dL	22

Cause of CKD (n=22)	
Diabetic nephropathy	11
Chronic glomerulonephritis	5
Hypertensive nephropathy	2
Lupus nephritis	1
Nephrotic syndrome	3
Mean Urine volume (mL)	1894 ± 847

Table 2: Comparison of Creatinine values (mg/dL) by Bland Altman analysis for fractional and conventional method and Effect of preservative on fractional urine collection method

Details of comparison	Mean difference (mg/dL)	Confidence Interval of Mean difference (mg/dL)		Limits of agreement (mg/dL)	Range (mg/dL)
		Lower bound	Upper bound		
U-24 h vs UF1	-5.658	-8.237	-3.079	-28.684 to17.368	13.000 to 258.350
U-24 h vs UF2	-6.018	-8.828	-3.207	-31.115 to 19.079	13.000 to 255.600
UF1 vs UF2	-0.360	-2.726	2.007	-21.487 to 20.768	13.000 to 255.750

U24h- 24h conventional urine collection, UF1-Fractional urine collection without preservative and UF2-fractional urine collection with preservative. All the samples are analysed after centrifugation.

Urine Creatinine results of fractional urine collection method without preservative (UF1) and with preservative (UF2) was compared to conventional method, ICC was 0.86 (C.I, 0.80 to 0.90) and 0.84 (C.I, 0.77 to 0.89) respectively. which is indicative of good correlation.

Table 3: Intraclass correlation between fractional urine and conventional urine collection

Conventional versus fractional method		Intraclass Correlation	95% Confidence Interval		P-value
			Lower Bound	Upper Bound	
U-24	UF1	0.86	0.80	0.90	<0.001
	UF2	0.84	0.77	0.89	<0.001

U24 -conventional urine collection, UF1-Fractional urine collection without preservative and UF2-fractional urine collection with preservative. All the samples are analysed after centrifugation.
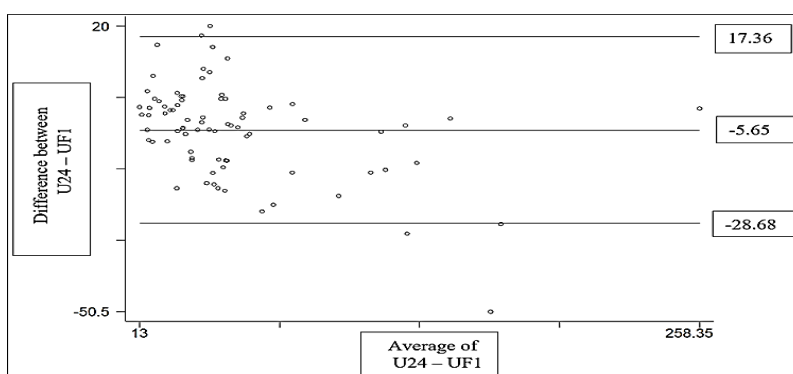
Fig 1: Bland-Altman comparison of UC-24 and UF1

U24h -24h conventional urine collection, UF1-Fractional urine collection without preservative and UF2-fractional urine collection with preservative. All the samples are analysed after centrifugation.

Limits of agreement (Reference Range for difference): -28.684 to 17.368mg/dL

Mean difference: -5.658 mg/dL (CI -8.237 to -3.079)
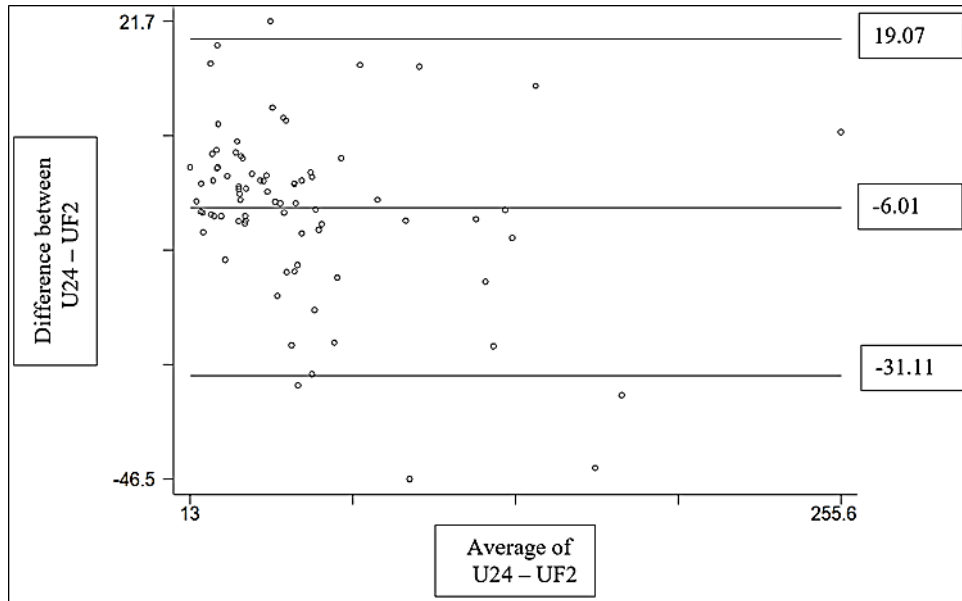
Range: 13.000 to 258.350 mg/dL

Fig 2: Bland-Altman comparison of UC-24 and UF2

U24h -24h conventional urine collection, UF1-Fractional urine collection without preservative and UF2-fractional urine collection with preservative. All the samples are analysed after centrifugation.
Limits of agreement (Reference Range for difference): -31.115 to 19.079 mg/dL
Mean difference: -6.018 mg/dL (CI -8.828 to -3.207).
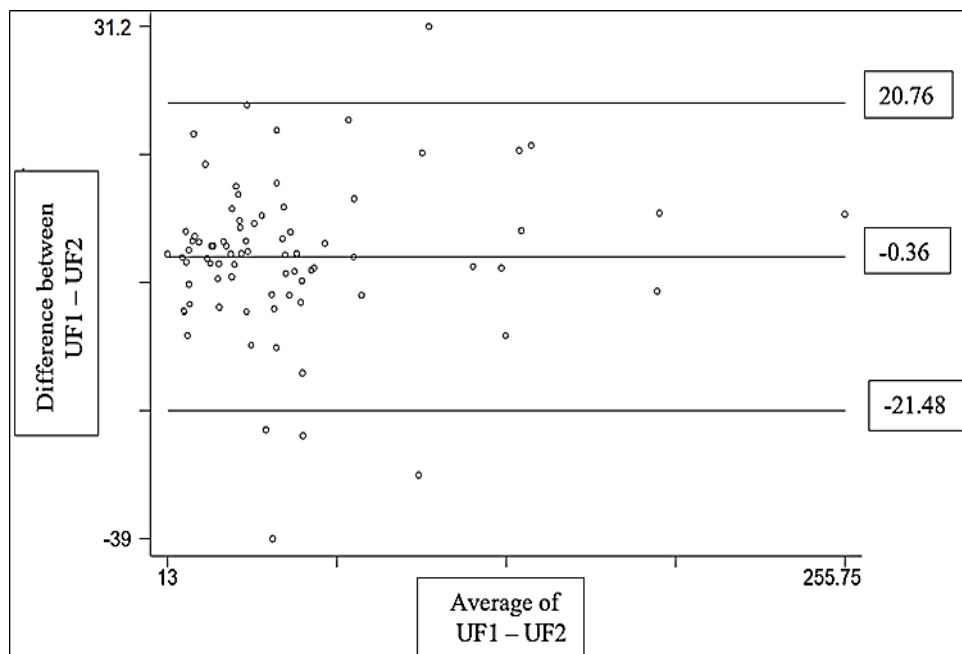Range: 13.000 to 255.600 mg/dL

Fig 3: Bland-Altman comparison of UF1 and UF2

U24h -24h conventional urine collection, UF1-Fractional urine collection without preservative and UF2-fractional urine collection with preservative. All the samples are analysed after centrifugation.
Limits of agreement (Reference Range for difference): -21.487 to 20.768 mg/dL
Mean difference: -0.360 mg/dL (CI -2.726 to 2.007).

Range: 13.000 to 255.750 mg/dL

Discussion

Creatinine clearance is one of the methods to asses GFR and   is required to diagnose and treat patients with renal failure. It is measured using urine volume (of 24 hours collection), urine creatinine concentration in 24 hour urine   and one time serum creatinine concentration[1]. Collecting the urine over 24hr period is cumbersome and tedious process. To overcome this there are several estimated (e-GFR) formulas to calculate[2]. There are limitations associated with these formulas [3]. In fact, study by Szymala-Pędzik M et al. showed estimated glomerular filtration (e-GFR) rate calculated with the formula gives incorrect classification of stage of chronic kidney disease [4].

Spot or random urine samples are tried as an alternative to 24h sample, which showed to be comparable as timed urine collections (complete collection of urine in large containers over set time period) for many analytes like urea, calcium and phosphorus, uric acid, microalbumin, protein and creatinine[5]. However, urine collection over 24hr period is a must to calculate creatinine clearance in few clinical settings such as patients having metabolic urinary stone diseases, estimation of renal function via creatinine clearance, proteinuria evaluation, estimating residual renal function in end stage renal disease, in patients with amputated limb and unusual body habitus including obesity, patients on chemotherapy regimens and during evaluations of potential kidney donors [6].

There are several studies that have attempted to compare shorter timed samples with 24h collection for protein excretion. We did extensive literature search to compare and evaluate with previous studies that followed similar fractional sampling for creatinine clearance estimation   but there seemed to be none. In fact, there are studies which compare the morning and random creatinine and 24h urine creatinine estimation which showed higher correlation to 24hr urine creatinine [5]. Studies [6-9] have been done comparing urine protein/creatinine ratio to 24hr protein to compare between random and 24hr sample and found to be comparable in few clinical settings.

To improve 24hr urine collection method our study attempted to make the urine collection patient friendly, by collecting fraction of urine (1 ml) during each void in entire collection period of 24hrs . To validate this method, we compared with conventional 24hr urine with hypothesis that both representative sample and whole sample will give similar urine creatine concentration and there by creatinine clearance . We also evaluated the creatinine values in conventional and fractional collection method with and without preservative.

In our study Mean difference of Creatinine in fractional collection with and without preservative was -0.360 mg/dl and confidence interval of mean with lower bound was -2.726mg/dL and upper bound was 2.007mg/dL which shows that the values are comparable between the two fractional collections. Urine creatinine values of both fractions (UF1 and UF2) compared to 24hr urine, showed similar results by bland Altman plot, indicated that both fractions were comparable even with difference in preservatives.

Interclass correlation coefficient between U24hr urine and UF1 and UF2 showed results of 0.86 and 0.84 indicating good correlation (Values above 0.75 indicates good correlation) and P value <0.001 indicates that there is no significant difference between the urine creatinine of two fractions and 24hr urine creatinine.

Our results have showed that with proper sampling the volume, urine creatinine can be measured by our proposed method since there is a good agreement with the conventional technique.

Limitations of the study

Sample size declined due to non-adherence of the study population to protocol and due to incomplete urine collection as per patient information.
The renal failure subjects were not tabulated separately. Hence comparability between the

fractions and 24hr urine could not be interpreted separately for renal failure patients. Representative sample collected by patients into small container was assumed to be equal amongst all the study subjects. However, this aspect would be difficult to verify.

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