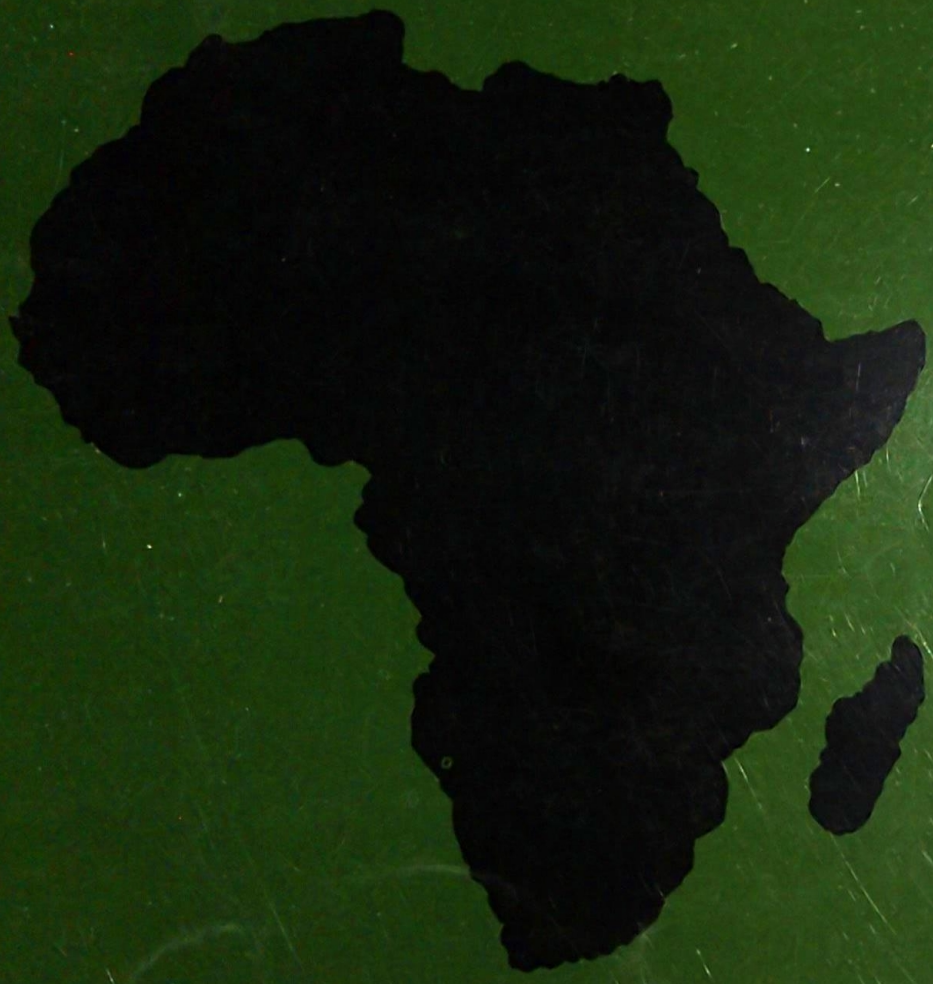
AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 30, NUMBERS 1 & 2, MARCH AND JUNE 2001



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ASSISTANT EDITOR:
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ISSN 1116 — 4077

Afr J Med med Sci (2001) **30**, 27 - 30

Creatinine clearance: alternative approach to traditional 24-hour urine collection in normal individuals

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Summary
Clinically, the commonly used routine test for assessing impaired renal function is the determination of creatinine clearance. The traditional 24 hour urine collection method is unreliable and inconvenient, particularly in ambulatory patients and outpatients because of errors in collection, timing of collection, and measurement of urine volume. The purpose of this study was to evaluate the possibility of determining creatinine clearance from urine collected for less than the traditional 24 hours. Thirty clinically healthy adult subjects with no history of renal dysfunction were used for the study. Each of the subjects had his creatinine clearance determined from 4 hour, 20 hour and 24 hour urine collections as well as from the formula of Cockcroft and Gault. The mean creatinine clearance obtained from 4 hour urine collection (male = 92.8ml/min/1.73m² & female = 84.5 ml/min/1.73 m²) and 20 hour urine collection (male = 98.9 ml/min/1.73 m² & female = 88.6ml/min/1.73 m²) shows no significant difference from that obtained from the traditional 24 hour urine collection (male = 97.9 ml/min/1.73 m² & female = 88.1 ml/min/1.73 m²) ($P > 0.05$). We therefore suggest that determination of creatinine clearance from fewer hours of urine collection especially in patients with renal impairment be explored towards their adaptation to routine practice.

Keywords: *Renal function; creatinine clearance determination; 4, 20 and 24 hour urine collections*

Résumé
En clinique, le tert de routine pour evaluer la fouction nenale, est la determination de la clarance de creatinine. La traditionalre deivine de 24hewies n'est pas satifaisante, et pas comode pour les patients ambulations, et les patients externes a cause des evaves de recotte, les heuse de recolte, et la mesure de volume. La portee de ette etude etait d'evoluer la possibilite de determiner la claviana de creatine dans l'wine recoltee pendant mouis que les traditionnellis 24 hewies. 30 adults cliniquement bieu portant san anteledant de troubles reinaux out ete utiliese pour ete etude. Chaviance de chaque sujet a ete evaluee pour la dewiere 4 heures, 20 heures et 24 heures, selon la fovnute de cockgoft et Gault. La claviana moyennae pour l'wire de 4 hewies (homes: 92.8ml/min/1.73m² et femwes=84ml/min/1.73m2 et pour l'wire de 20 hewies = 98.9ml/min/1/73m3 femmes=88.6ml/min/1.73m2) n'out moutre aucune difference significative,de l'wire de 24 hewies. (Homues = 97.9ml/min/1.73m2 et femmes = 88.1ml/min/1.73m2) $P > 0.05$). Nous proposons aiusi que la determination de la chairance de creatinine fairte sur quelques heweis, speciallement chez les patients insuffisant renaux sort consideree en vue etre adoptee dans la pratique qustidienue.

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Introduction

Based on the theory of "intact Nephron hypothesis" assessment of Glomerular Filtration Rate (GFR) by determining the creatinine clearance has become a prominent investigative tool for evaluating renal function in clinical practice. From procedural point of view, two factors influence measurement of creatinine clearance and thus its correct interpretation. First, the most common methods for measuring creatinine employ the non-specific alkaline picrate reaction and thus non-creatinine chromogens in plasma increase the apparent plasma concentration by as much as 30% if serum values are <530 Fmol/L and by approximately 10% if values are >530Fmol/L. The percent increase is progressively less with higher serum creatinine concentrations[1]. (Urine contains considerably less non creatinine chromogens). This overestimation of plasma creatinine concentration results in an underestimation of creatinine clearance and partially offsets the apparent high clearance of creatinine that is due to tubular secretion. As a result, the endogenous creatinine clearance, by coincidence, agrees closely with the inulin clearance over a substantial range of clearances [2].

Second, and a persisting problem is the accuracy of the traditional 24 hour urine collection for the measurement of creatinine clearance which depends on a meticulous urine collection which actually is not possible without carrying out bladder catheterization. Due to this inadequacy and other difficulties in collection of urine like timing of collection measurement of volume, an error of about 20% has been noted in the determination of creatinine clearance using a 24 hour urine collection [3].

The persistent problem has led to the use of different approaches which are based on disappearance from serum of a radio isotope labelled substance solely eliminated by glomerular filtration and/or the use of derived formula for predicting creatinine clearance from serum creatinine and other variables (including age, height and body weight) which are known to affect the creatinine clearance. The main drawback of this alternative approach is the cost of equipment and difficulty with sourcing constant supplies in the Nigerian setting. Also important is the problem of storage, and safety in handling of radioactive substances.

The possibility of obtaining a significant value from 4 hour urine collection has been mentioned [4], this study was therefore designed to evaluate the possibility of determining creatinine clearance from 4 hour and 20 hour urine collections as well as from the formulae of Cockcroft and Gault [5].

Materials and methods

Study group

A total of 30 clinically healthy adult subjects with no medical history of renal impairment were used for this study, after obtaining the approval of the research, and ethical committee. 19 of them were males and 11 were females, they were all

members of the University of Ilorin community. Informed consents were obtained from all the subjects. Those with history or clinical evidence of renal impairment were excluded. The subjects were not on drugs such as cimetidine, co-trimoxazole and diuretic which are known to affect creatinine clearance. Fluid was allowed freely. The height (centimetres), age, (years) and weight (kilograms) of each subject were recorded for surface area determination from nomogram.

Collection of blood sample and treatment

Ante cubital vein area was disinfected with methylated spirit and 5ml of venous blood was collected at about the mid-point of the 24 hours urine collection period for the sake of consistency.

The blood sample taken into a plain specimen bottle was first allowed to clot and retract before it was centrifuged for 10 minutes at 2500-3000 r.p.m. Serum was separated into clean covered serum bottles and frozen immediately at -20°C till the following day when analysis could not be done the same day.

Collection of urine sample and treatment

The subjects were given two 2-litre plastic containers each. One for the 4 hour and the other for the 20 hour urine collection after the procedure for collection of Rock and Walker *et al*[1]. The first 4 hours of the urine collection period was taken as the 4 hour period in one of the 2 litre bottles, and the collection was continued for further 20 hour in the other 2 litre plastic bottle to complete the 24 hour period. Subjects were given a funnel each for proper collection and were asked to report any urine loss or error in collection.

The volume of the 4-hour and 20 hour urine samples were measured while that of 24 hour urine was calculated. Aliquots of the 4 hours and 20 hour urine were taken. The two were later mixed and an aliquot taken for the 24 hours sample. This was done to remove possible problems that daily variations could have brought on the result of this study. All Aliquots were frozen at -20°C overnight when analysis was not done immediately.

Determination of creatinine

This was done using the alkaline picric acid method otherwise known as the Jaffe reaction[7]. The principle of the method is that creatinine gives a red colour with alkaline solution of picric acid which is measured colorimetrically at 520nm. The samples were stored frozen overnight when analysis were not done immediately. The samples, both urine and blood, were allowed to thaw and equilibrate with room temperature before assayed.

The clearance was calculated using the formula

$$C_{cr} = \frac{U_{cr} \times V_{(ml)}}{S_{cr} \times h \times 60} \times \frac{S}{1.73_{(min)}}$$

(ml)

where C_{cr} = creatinine clearance

U_{cr} = concentration of creatinine in urine (Fmol/l)

V = volume of urine in millilitre (ml)

S_{cr} = concentration of creatinine in serum (Fmol/l)

h = The period of collection of urine in hours

S = surface area of the subject

The surface area of the subjects was determined from nomogram by using their heights in centimetres and weight in kilogram that were previously recorded.

Cockcroft and Gault formula

$$\frac{(140 - \text{Age}) \times \text{weight}}{\text{Scr} \times 72} \text{ - for male}$$

$$\frac{(140 - \text{Age}) \times \text{weight}}{\text{Scr} \times 85} \text{ - for female}$$

Statistical analysis

Statistical analysis was carried out in an IBM-compatible Personal Computer using EPI info version 6.1 which is a database and statistical software developed by the Centre for Disease Control, Atlanta, Georgia, United States of America. A one-way analysis of variance was carried out on the creatinine clearance values. After establishing that the variables conform with normal distribution: a further evaluation of the variables was done in which the student t-test was used to determine the level of significance for the mean values of creatinine clearance of the 4 hour, 20 hour and 24 hour urine collection and also for the creatinine clearance obtained through formula. The statistical significance was accepted at the $P<0.05$ level.

Results

Thirty clinically healthy subjects were used for this study. None of them reported any loss or error during urine collection. Details of their mean ages, mean weight, mean height, mean value of serum creatinine concentration and sexes are displayed in Table 1. Table 2 shows the mean urine volume, mean urine creatinine concentration and mean creatinine clearance for 4 hour, 20 hour and 24 hour urine collections.

Table 1: Mean age, weight, height and serum creatinine concentration

	Male	Female
Mean Age years (SD)	25.63 (3.02)	23.36 (2.06)
Mean weight kg (SD)	62.0 (6.15)	59.5 (7.02)
Mean Height cm (SD)	168.97 (9.00)	161.09 (8.13)
Mean serum creatinine concentration Fmol/L (SD)	123.6 (6.13)	113.3 (5.46)

Table 2: Mean values for urine volume and urine creatinine concentration

	4 hrs		20 hrs		24hrs	
	Male	Female	Male	Female	Male	Female
Mean Urine (ml) (SD)	218.6 (74.8)	205.7 (42.9)	1003.7 (314.2)	1077.5 (190.6)	1221.2 (346.9)	1282.5 (193.6)
Mean Urine creatinine concentration (Fmol/L)	13903.1 (4650)	10612.5 (3778)	15892.8 (59078)	11401.6 (16769)	15498.9 (5676.9)	11333 (14389)

Endogenous creatinine clearance

Table 3 shows the analysis of variance of the mean values of the various creatinine clearance results. This shows that lumped together there is no statistically significant difference among the values (F-0.066). However, a further evaluation with student-test is recorded in Table 3.

Table 3: Analysis of variance for mean creatinine clearance values

Source of varaince	Degree of freedom	Sum of square	Means square	Variance ratio	F
Time	3	25,529	510.6	1.266	0.066
Residual	108	43552.32	241.98		
Total	111	69081.32			

Table 4 shows the mean creatinine clearance, range of clearance, standard error of the mean, standard deviation and P-value for the 4 hour, 20 hour and 24 hour urine collections and also that of formula. The mean creatinine clearance from 24 hour urine collection of 97.9ml/min/1.73m² for male and 88.1 ml/min/1.73m² for female served as the reference values against which other values are compared.

For the 20 hour urine collection in both sexes, the mean creatinine clearance values of 98.9 ml/min/1.73m² for male and 88.6 ml/min/1.73m² for females are not significantly different from the 24 hour mean creatinine clearance. The mean creatinine clearance values for 4 hours urine collection in both male and female were found to be 92.8 ml/min/1.73m² and 84.5 ml/min/1.73m², respectively. Values of $P>0.05$ (male) and P>0.05 (female) were obtained when compared with values from 24 hour urine collections.

However, the creatinine clearance values of 70.5 ml/min/1.73m² for male and 63.6 ml/min/1.73m² for female subjects obtained from the use of the Cockcroft and Gault [5] were significantly different from the mean creatinine clearance from 24 hour urine collection with *P*-value of $P<0.001$ for male, and $P<0.001$ for female subjects.

Table 4: Mean creatinine clearance

	24hr urine collection		20 hr urine collection		4 hr urine collection		formula	
	Male	Female	Male	Female	Male	Female	Male	Female
Mean creatinine clearance (ml/min/ 1.73m²SD)	97.9 (16.99)	88.1 (11.98)	98.9 (18.13)	88.6 (12.90)	92.8 (17.91)	84.5 (10.76)	70.5 (9.18)	63.6 (6.84)
2 SEM	7.79	7.10	8.78	7.78	8.22	11.00	4.21	4.13
Range	97-137	71-114	75-144	72-120	63-143	64-97	50-91	56-80
P-value	>0.05	>0.05	>.05	>.05	>.05	>.05	<.001	<.001

Discussion

Following the success of Miller and Winkler in 1938, the clearance of endogenous creatinine became widely applied in clinical and scientific work as the standard procedure for the assessment of glomerular filtration rate [3]. Several authors have compared creatinine clearance with glomerular filtration rate (GFR) measured as inulin clearance. While a few found a close approximation [8,9,10,11], most have demonstrated that endogenous creatinine clearance is an inaccurate estimate of GFR [12,13,14,15,16,17,18].

In normal (healthy) individuals as a result of low serum creatinine concentration the contribution of the non-creatinine chromogens cancels out the adverse effect of tubular secretion on the use of creatinine clearance for assessing GFR [2.19], thus leaving only the procedural problems associated with difficulties in collection of urine, like timing of collection, measurement of volume which Miller and Winkler reported to account for an error of up to 20% in the calculation of creatinine clearance value.

In an attempt to reduce the error associated with the traditional 24 hour urine collection, Richardson and Philbin reported a good correlation between creatinine clearance calculated from 1 hour urine collection [20] while Odutola and Adoun also proposed calculation from 4 hour urine collection from their preliminary report [4]. This study is in agreement with the works of the above mentioned workers that there is no statistically significant difference in the creatinine clearance obtained from 4 hour urine collection when compared with that of 24 hours. Also this study shows that the creatinine clearance obtained from 20 hour urine collections (98.9ml/min/1.73m² male and 88 ml/min/1.73m² female) demonstrated no statistically significant difference when compared with that obtained from 24 hours with *P*-value >0.05.

However, there was a significant difference in the mean creatinine clearance of both male and female subjects when the values obtained in the formula calculation was compared with the 24 hour urine collection using the Cockcroft and Gault formula ($P<0.001$ for males and $P<0.001$ for females). This is in agreement with the conclusion of Taylor et al [21] who also observed that formula alone is not a good measurement of creatinine clearance. Many workers [10,22] have since suggested that pitfalls like differences in the quality and quantity of protein intake will affect the weight of the subjects even if they had the same quantity of serum creatinine. This study considering the heterogenous background of the people in the university community agrees with this line of reasoning.

In conclusion, this study shows that deriving creatinine clearance from formula alone is not adequate. Creatinine clearance obtained from 4 hour and 20 hour urine collections in clinically healthy subjects do not show any statistically significant difference from the value obtained from the traditional 24 hour urine collection. It is therefore being recommended that urine collection for a shorter period with its attendant cost effectiveness and convenience be considered for further studies.

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