Final report

Study on spot urine osmolality: creatinine ratio in healthy humans

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Co-investigators : Dr Srini Godevithane
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Grant number: RG/2004/M/12

Department of Anaesthesiology
Faculty of Medicine
University of Peradeniya

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Section one

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Faculty of Medicine
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(Institution, degree and dates) : Not registered for post graduate degree

Section two

Synopsis

SPOT URINE OSMOLALITY: CREATININE RATIO IN HEALTHY HUMANS

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Spot urine albumin/ creatinine ratio is a reliable estimate of 24-hour urine albumin excretion since it accounts for the variability in urine concentrations over time. Similarly urine osmolaliy: creatinine (Osm/cr) ratio of a spot urine sample may give an overall estimate of urinary excretion of solutes, renal concentrating ability and body hydration status.

We used a pilot study to determine whether spot urine Osm/cr is a reliable indicator of body hydration status in healthy individuals. Pilot study concluded that spot urine Osm/cr in healthy adults is a reproducible entity in steady state and it is a consistent urinary index in steady state.

The objective of the second study thereafter was to establish spot urine Osm/cr in healthy humans and its variation in relation to gender, age, body weight and height.

Two hundred and thirty two healthy volunteers participitated.They were stratified to 7 age groups.i.e.(a)1.5-5years,(b)>5-10years,(c)>10-20years,(d)>20-

30years.(e)>30to45(f)>45- 60years.(g)>60years.15 males and 15 females were allocated for each age category. A spot urine sample was collected from all patients and was analyzed for urine osmolality and creatinine in batches of 50.

The influence of age, sex, body weight and height on the urine Osm/cr was analyzed using multiple linear regression and only height showed a significant correlation. R square was 0.02 suggesting that height may make 2% influence on urine Osm/cr.Further analysis after excluding 1.5 to 5 year age group reveal no significant correlation between age,sex,body weight and height with Osm/cr ratio

The study concluded that urine Osm/cre ratio need no correction for gender, age and body weight above the age of 5 years.

Section three

I. Introduction:

Background and justification.

Spot urine albumin/creatinine ratio is a reliable estimate of 24-hour urine protein excretion since it accounts for the variability in urine concentration over time[1, 2]. Urine uric acid/creatinine, urine calcium/creatinine ratio also have been used to estimate urinary excretion of those substances[3] Similarly urine osmolality /creatinine (Osm/cr) of a spot urine sample may give an overall estimate of urinary excretion of solutes and renal concentrating ability. Renal concentrating status at a given time is dependant on the ability of the kidney to concentrate and body fluid status. In the steady state urine excretion of creatinine is assumed to be a constant. Therefore urine osmolality: creatinine ratio may give an estimate of renal function and body hydration status. Whether the ratio correlates with body hydration status, however, has not been established.

Pilot study

We used a pilot study to determine whether spot urine Osm/cr is a reliable indicator of body hydration statuses in healthy individuals.

Specific objectives of the pilot study

1. To establish the consistency of Osm/cr ratio in spot urine sample in healthy humans on repeated measurement and
2. To establish behavior of Osm/cr ratio in spot urine sample with water loading in healthy humans
3. To establish behavior of urine Osm/cr ratio in abnormal state for example in mechanically ventilated patients with organophosphate poisoning.

The study population

The present pilot study was conducted on there population groups.

1. From university student population a group of 30 healthy volunteering medical students ,University of Peradeniya
2. From university student population group of 21 healthy volunteering medical students ,University of Peradeniya
3. Organophosphate poisoned patients who were on mechanical ventilation in the intensive care units of Teaching Hospitals Peradeniya and Kandy.

Results of pilot study

Early morning spot urine samples of 1.5ml were collected from 30 healthy volunteers over four consecutive days and urine osmolality and creatinine concentration was measured. Mean Osm/cr ratio was 66.6mosmolkg^{-1}/mmoll^{-1}
(Mean osmolality =534.0mosmol.kg^{-1}, mean creatinine=9.89mmoll^{-1}). On repeated measurement of consecutive urine sample a constant mean Osm/cr ratio was evident over four consecutive days.

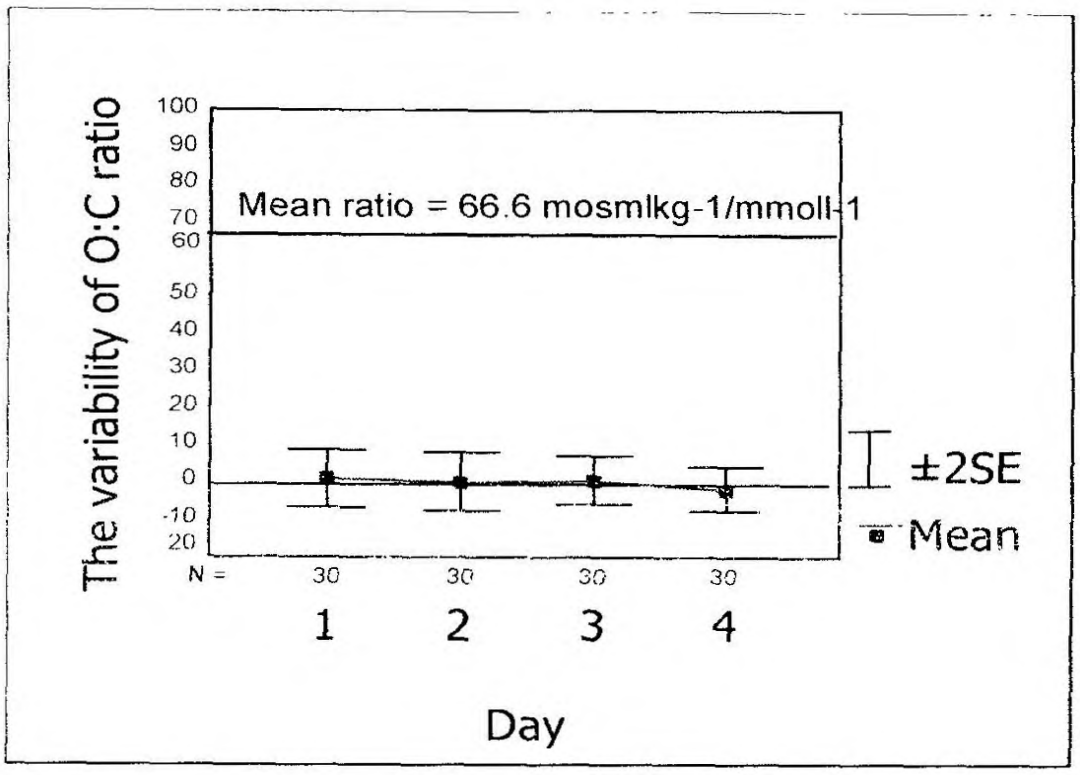

Fig 1: The variation of spot urine Osmolality: creatinine ratio in healthy humans over four consecutive days. (Bland – Altman plot)

Table 1: Mean spot urine Osmolality: creatinine ratio in healthy humans over four consecutive days.

Day	Osmolality:creatinine ratio Mean(SD) mosmolkg-1
1	66.6(5.29)
2	65.9(4.71)
3	66.6(4.27)
4	64.0(4.12)

Thus Osm/cr was found to be relatively constant and reproducible on repeated measurements in steady state.

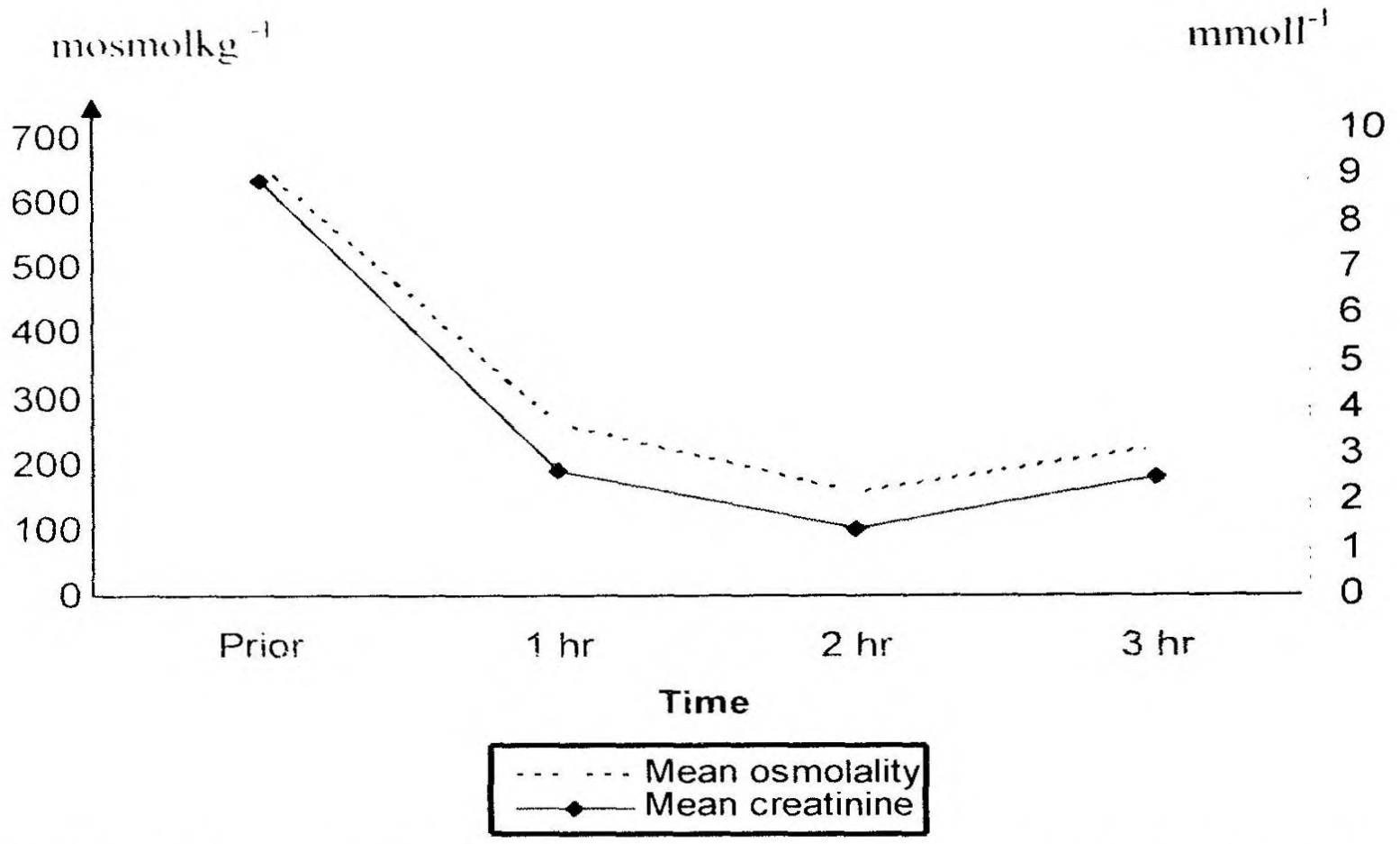

Fig 2: The urine osmolality and creatinine concentration with water loading in healthy humans.

Another 21 normal individuals were subjected to a simple water loading test by giving 1.0 liter of water to drink, and urine samples were collected prior to and 1, 2, 3 hours after commencing water loading. Their urine osmolality and creatinine concentration were analyzed.

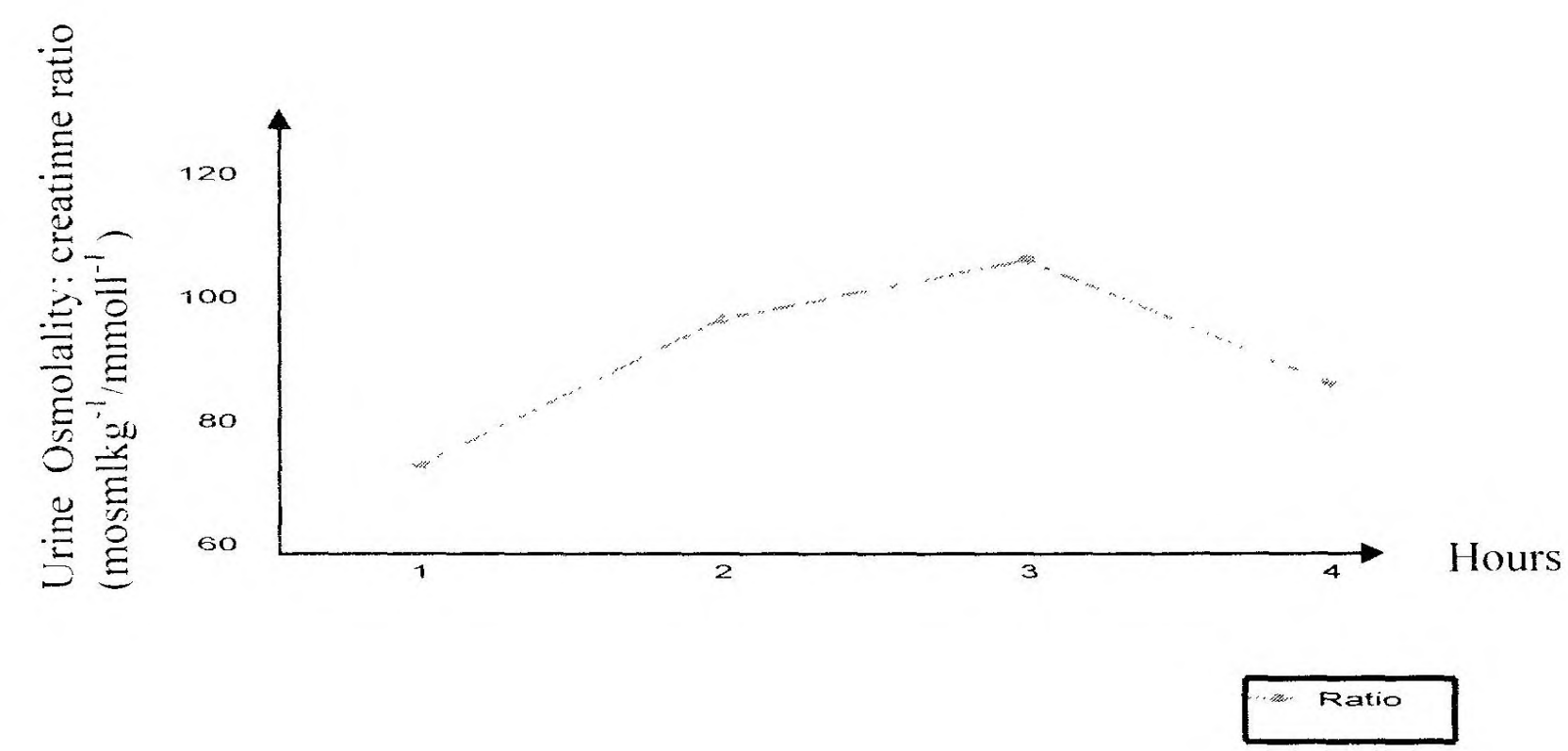

Fig 3: Urine Osmolality: creatinine ratio with water loading in healthy humans.

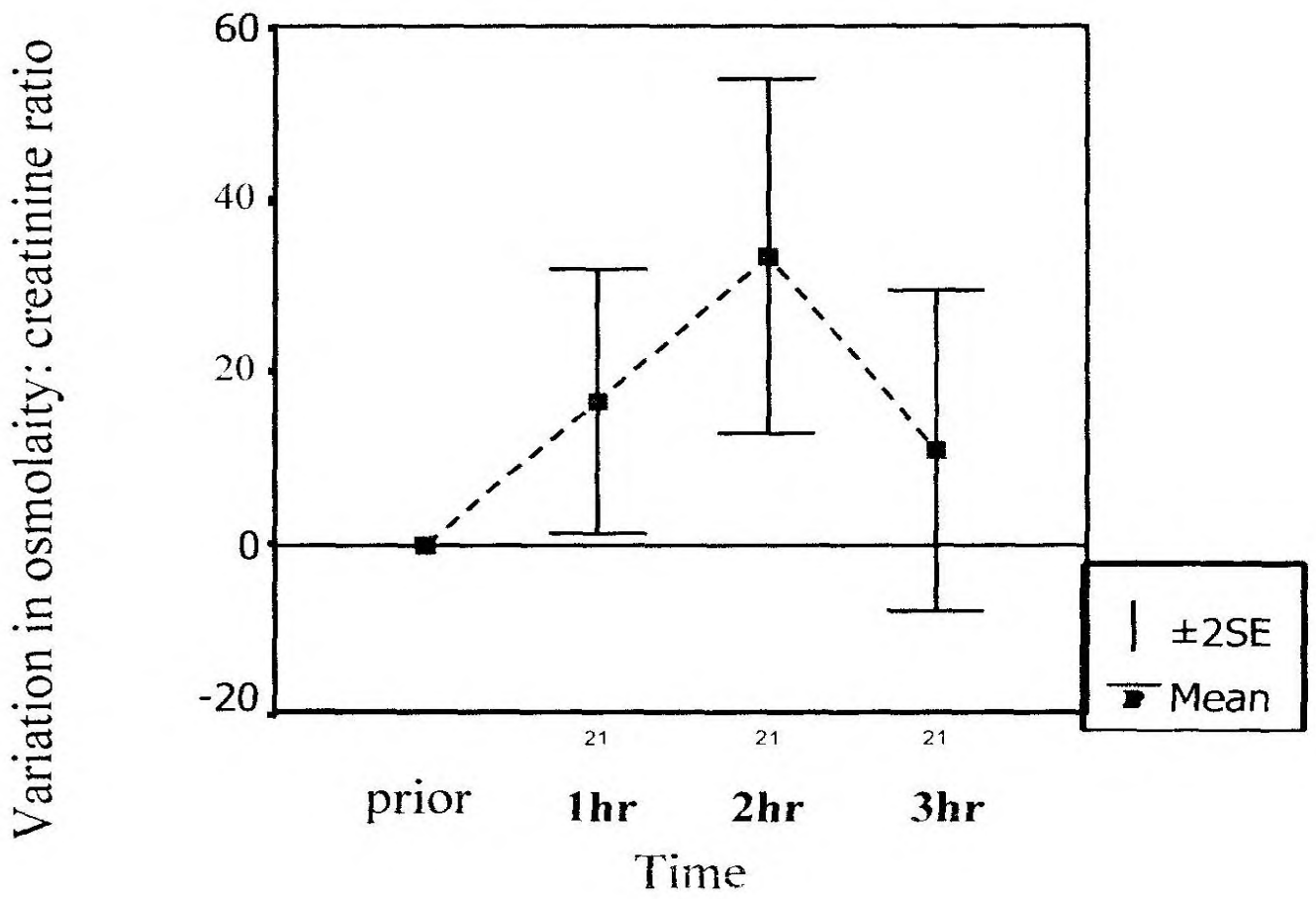

Fig 4: The variation in spot urine osmolality: creatinine ratio from baseline with water loading

Osm/cr was found to be increasing with dilution of urine with a significant change at 2 hours after drinking water. (P=0.02, 95% CI =64.03 to – 3.13)

Further study was carried out by collecting catheter urine samples of organophosphate poisoned patients admitted to ICU for mechanical ventilation on 5^{th} to 9^{th} day of poisoning. It shows significantly higher Osm/Cr on 4 consecutive days.

The study concluded that urine Osm/cr ratio is a reproducible entity and it is a consistent urinary index in steady state in healthy humans. It was also noted that it would be abnormal in patients with acquired diseases.

Our second study therefore designed to establish the influence of age, sex body weight and height on spot urine Osm/cr in healthy humans.

II. Objective

To establish spot urine Osm/cr ratio in healthy humans and its variation in relation to gender, age, body weight and height.

III. Materials and method used including statistical analysis.

The study was conducted on healthy volunteers. Subjects with history of hypertension, diabetes mellitus, chronic renal disease or with urinary tract infection and who were on medication that may interfere with renal creatinine excretion such as cimetidine, trimethoprime, dapsone,nephrotoxic drugs, diuretics were excluded. Thereafter, the selected population group categorized according to the age.

1.5 months to 5 year old healthy children were recruited from two local clinics conducted for routine vaccinations and 5-18 years old group of healthy children and adolescents from local schools and 18 to 30 age healthy subjects from university resident students and

others from local village people .Subjects with even number of birth dates were only selected.

Written informed consent was obtained from each subject prior to the study having explained the purpose of the study and the extent of their involvement. Research protocol was approved by the ethics committee of the Faculty of Medicine, University of Peradeniya.Spot urine samples of 1.5ml were collected into plastic containers and stored in eppendorf tubes at -20 ^{0}C within 2 hours of collection. Samples were analyzed in batches of 50.

Age,sex and body weight was recorded on a standard form. All subjects above 2 ½ years of age were weighed using a bathroom scale to approximate 1kg.The children below 2 ½ years were weighed using a eye level physician beam scale. The same scales were used throughout the study by the same observer.

Urine creatinine concentration was determined by kinetic Jaffe reaction [4] (Alkaline pirate solution) using auto analyzer.Autoanalyzer was calibrated with standard solutions before each batch.Urine osmolality was measured by freezing point depression method [4] via micro-osmometer.Osmometer was calibrated with distilled water and standard solutions before each batch of samples. Two urine samples, one with high osmolality and the other with low osmolality was repeatedly analyzed within each batch and between batches to calculate inter and within assay variation.

Table 2: Within and inter assay variation of osmolality and creatinine measurement

	Within assay variation Mean (SD)	Inter-assay variation Mean (SD)
Osmolality (mosmol/kg)	1.51 %(1.70)	1.24 %(1.45)
Creatinine (µmol/dl)	1.05 %(0.37)	1.92% (0.57)

The influence of gender, age, body weight and height on the Osm/cr was assessed using multiple linear regression and SPSS 10.

IV. Results/observations

Study was conducted in 232 healthy volunteers.

Table 2: Age distribution of the sample analyzed.

Age Category	1.5-5	>5-10	>10 -20	>20-30	>30 -45	>45 -60	>60
Female	17	17	19	24	14	18	3
Male	17	26	33	15	11	9	6

Table 3: The mean Osmolality: creatinine Ratio in each age category.

Age category	Female Mean ±SD	Male Mean ±SD
1.5 to 5	208.32 ± 92.47	219.16 ± 127.98
>5 to 10	139.67 ± 55.60	130.41 ± 29.56
>10 to 20	83.36 ± 16.36	98.41 ± 39.32
>20 to 30	62.08 ± 22.51	73.37 ± 24.45
>30 to 45	104.42 ± 33.59	66.87 ± 26.38
>45 to 60	125.41 ± 58.38	52.50 ± 16.75
>60	111.03 ± 15.62	59.11 ± 18.08

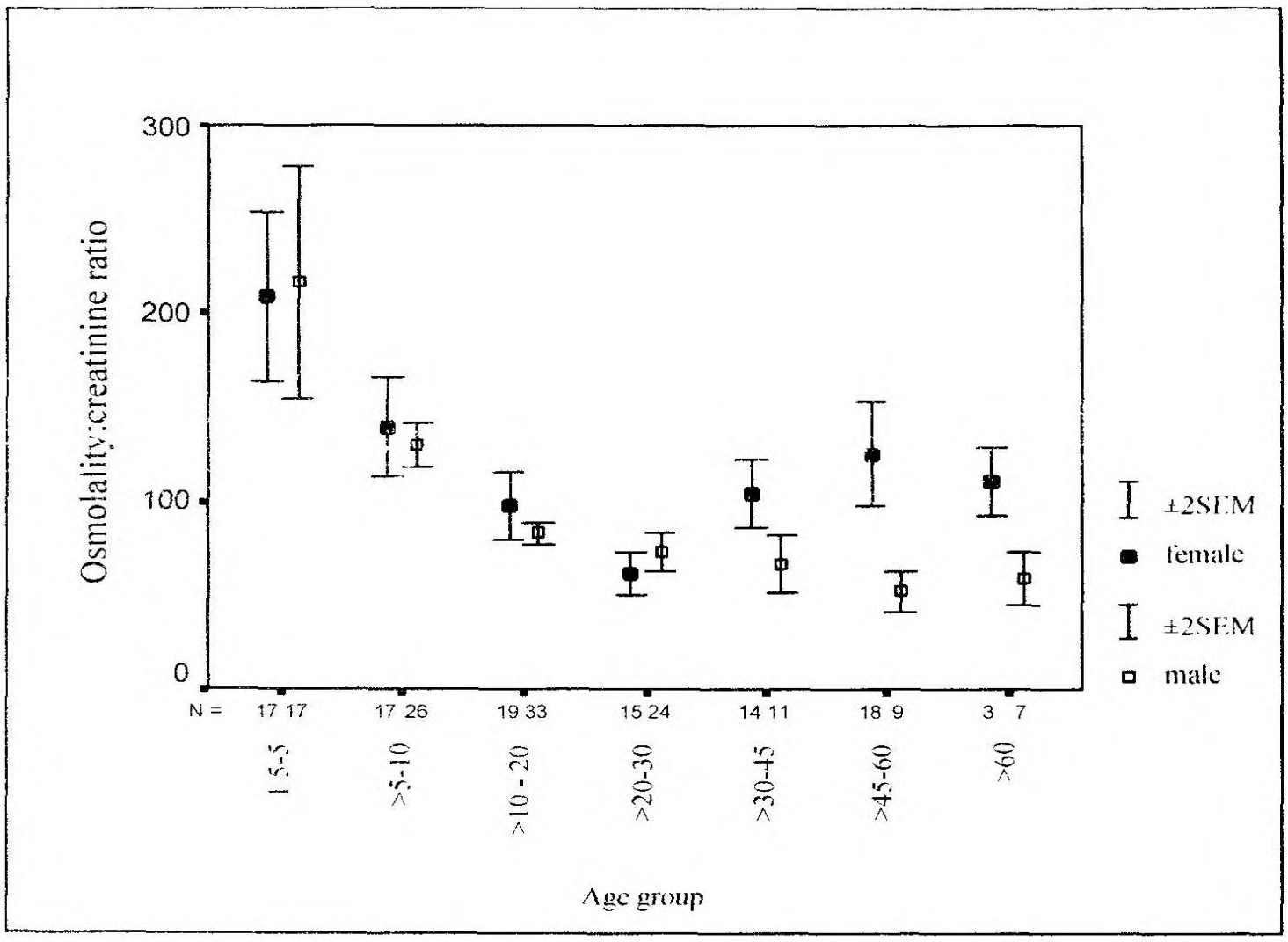

Fig 5: Spot urine osmolality: creatinine ratio in each age category in healthy humans.

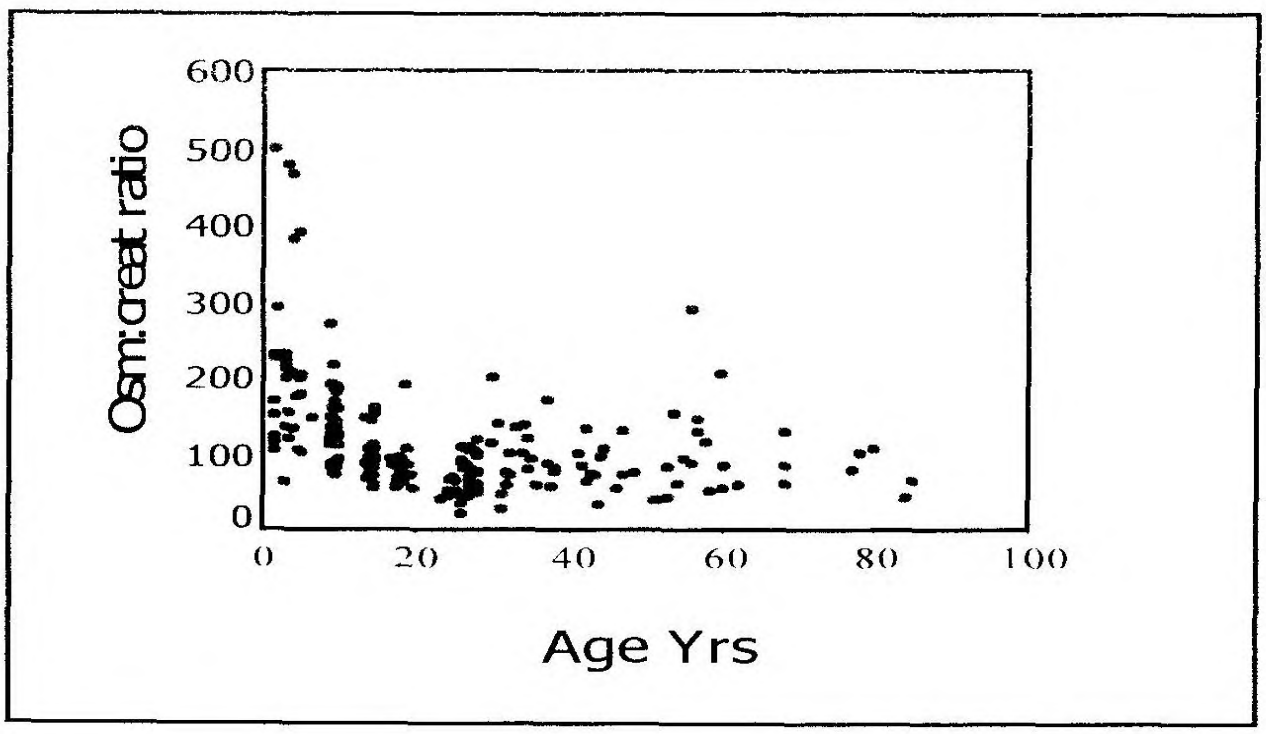

Fig 6: A Scatter plot of urine osmolality: creatinine ratio and age

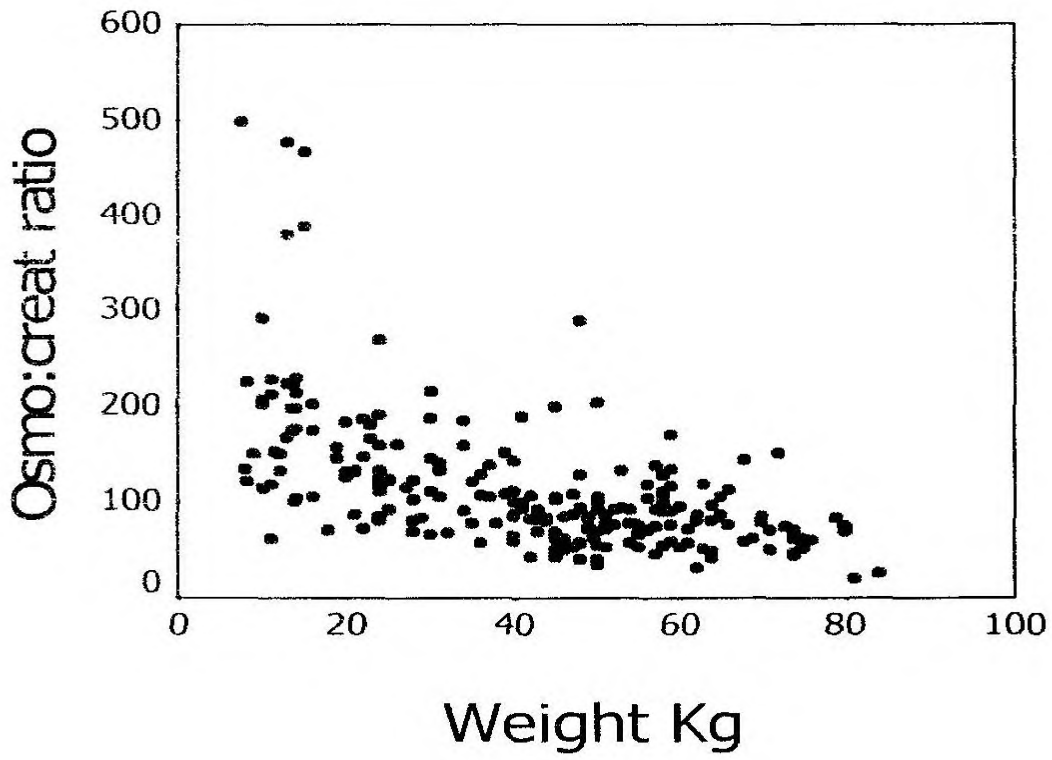

Fig 7: A scatter plot of spot urine osmolality: creatinine ratio and body weight

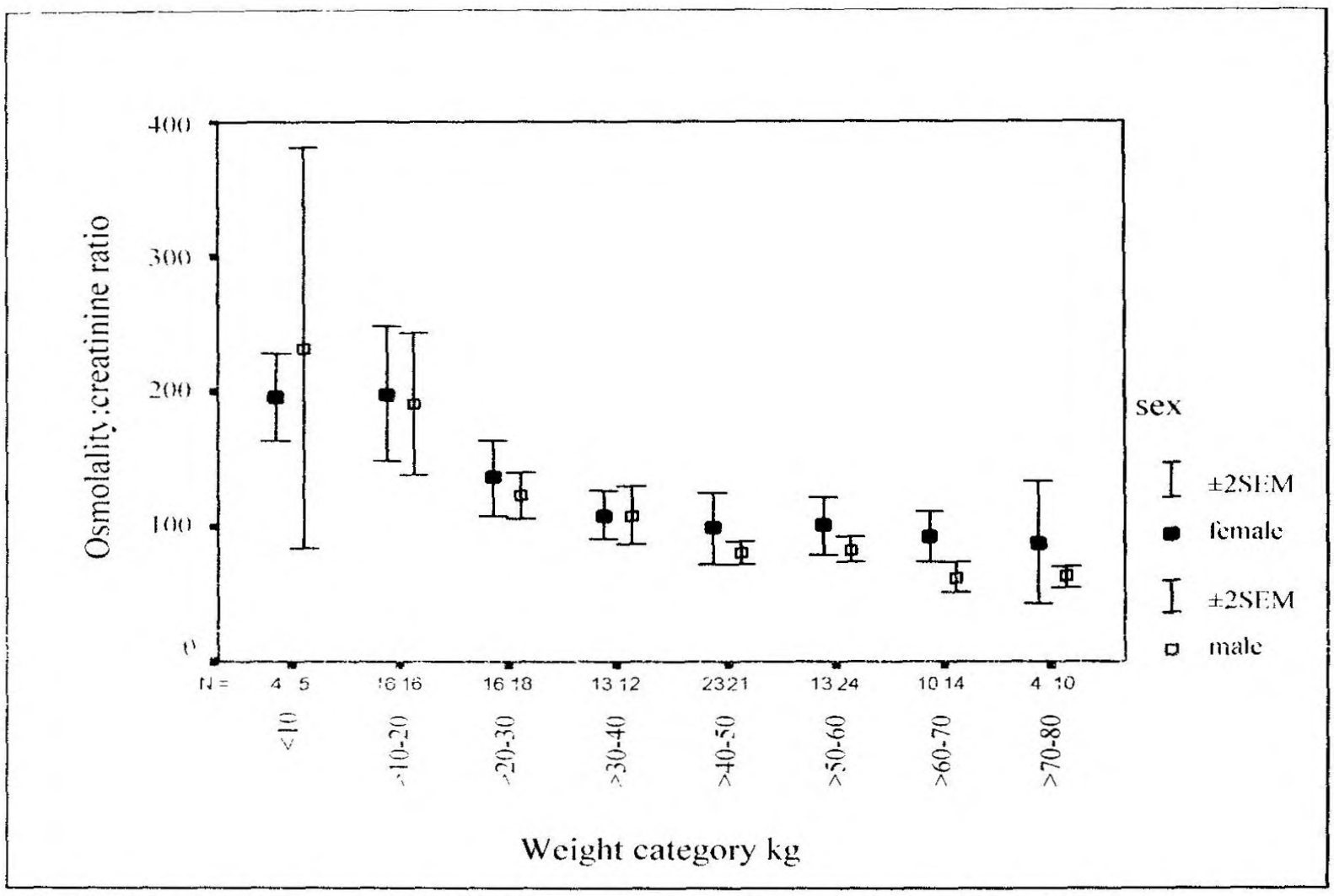

Fig 8: Spot urine osmolality: creatinine ratio and body weight

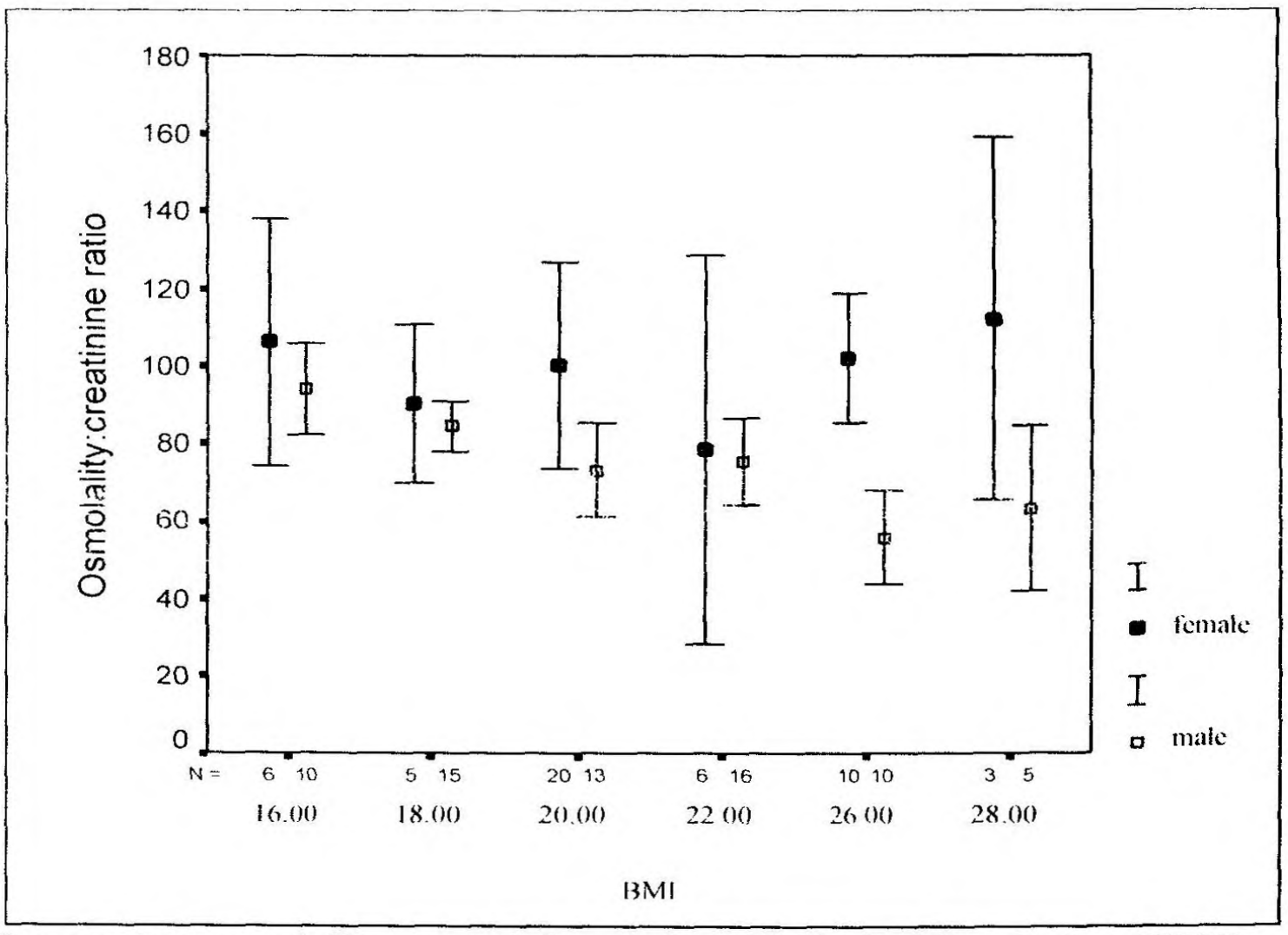

Fig 9: Spot urine osmolality: creatinine ratio with Body Mass Index (BMI)

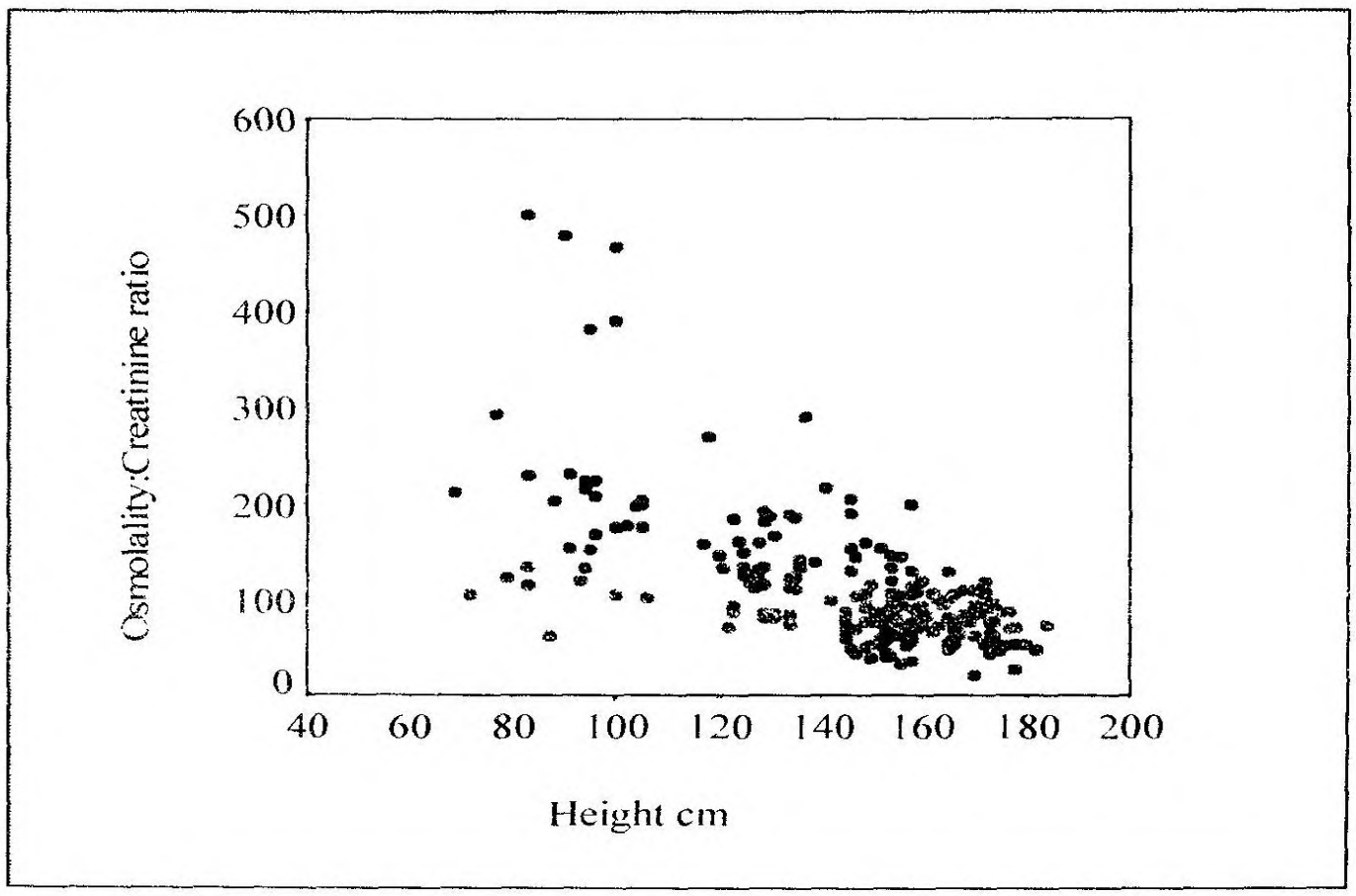

Fig 10: A scatter plot of spot urine osmolality: creatinine ratio with height

V. Discussion

Urine osmolality measures the concentration of osmotically active particles in urine. Body hydration status and concentrating ability of the kidney contributes to the urine concentration and thus urine osmolality can yield important information about patient's ability to maintain abnormal fluid balance status. Therefore measurement of urine osmolality may help to establish a diagnosis and predict the fluid status of the body. but requires the measurement of concentration of the urinary solute in timed urine collection.

However twenty four hour urine collections are commonly used to smooth the wide fluctuation in the osmolality over the day, but are time consuming and often imprecise due to error related to urine collection .An alternative approach avoiding timed or twenty four hour urine collection is single spot urine specimen standardizing with urinary creatinine concentration since the creatinine excretion is a constant in steady state. In

patient with diabetes mellitus protein: creatinine ratio of spot morning urine sample is a reliable indicator of 24 hour urinary protein excretion.[1] Similarly osmolality: creatinine ratio of spot urine sample may reflect rate excretion of osmotically active particles in urine.

However production of creatinine is related to lean body mass. which generally does not change within relatively short period of time.[7,8] When creatinine concentration are constant, the glomerular filtration rate is stable and tubular secretion and reabsorption of creatinine (in health at most age) is minimal, the rate of elimination of creatinine from the body does not fluctuate very much. This steady state is the rationale for using urinary creatinine concentration as a reference standard for the chemical analysis of spot urine.[9] Since lean body mass is varied with age and gender of an individual it may have an effect on Osm/cr ratio.

Therefore our study was set out to see the influence of age. sex, body weight and height on spot urine Osm/cr in healthy humans.

The results clearly shows greater scatter in the urine Osm/cr ratio values during the early years of life with higher values of standard error of mean Osm/cr.This is in contrast the picture seen in older children and adults where Osm/cr ratio remain relatively constant with less scatter.

Multiple linear regression statistics were calculated. Among the variables tested by us only body height showed significant correlation with Osm/cr.No significant correlation between age. sex and body weight with Osm/cr.R square was 0.02 suggesting that height may make 2% influence on urine Osm/cr .Since there is more variability in Osm/cr ratio further analysis was done using multiple liner regression after excluding 1.5 to 5year age group. No significant correlation was observed between age. sex. body weight and height with Osm/cr.This is probably due to difference in tubular handling of creatinine in younger age group compared to older children and adults with the maturation of kidney.

What ever the pathophysiological explanation for changes in spot urinary Osm/cr ratio in young, above observations guide us to conclude that spot urinary Osm/cr ratio need no correction for gender, age body weight and height in those who above the age of 5 years

Thus spot urinary Osm/Cr ratio measurement is at least as reliable as 24 hour urinary osmolality predicting renal concentrating ability and assessing body hydration status in older children In addition it is easier to perform, less time consuming sensitive and relatively inexpensive. [5-6]

VI. Conclusion drawn and recommendations

Spot urinary osmolality: creatinine ratio needs no correction for gender, age, body weight and height above 5 years of age.

VII. Future work stemming from conclusions

Measurement of urine osmolality/creatinine ratio may help to establish a diagnosis of renal disease and predict the fluid status of the body,

VIII. References

1. Bakker AJ: Detection of microalbuminuria receiver operating characteristic curve analysis favors albumin: creatinine ratio over albumin concentration. Diabetes Care 1999; 22:307-13.

2.Ruggenenti P.,Gaspari F.,Perna A.,Remuzzi G:Cross sectional longitudinal study of spot urine protein :creatinine ratio ,24 hour urine protein excretion rate ,glomerular filtration rate and end stage renal failure in chronic renal disease in patients glomerular filtration ,and end stage renal disease in patient without diabetes.BMJ 1998;317(7171):1491.

3. Guimaraes M.G.M., Santos J.S., Moreira M.M. et al: Urinary excretion of calcium, uric acid and citrate in healthy children and adolescents.J.Pediatr 2002; 78(2):153-160.

4. Bland J.M., Altman D.G: Statistical methods for measuring agreement between two methods of clinical measurement. Lancet 1986; 1(8436):307-310

5. Kaufman J.M ., Martin L.G., Seegmiller J.E: Urine uric acid to creatinine ratio. A screening test for inherited disorder of purine metabolism J Pediatr 1968;(4):583-92.

6. Ginsberg J.M., Chang B.S., Matrese R.A., Garella S: Use of single voided urine sample to estimate Proteinuria.N Eng J med 1983; 309:1543-46

7. Miller A.T., Blyth C.S.,: Estimation of lean body mass and body fat from basal oxygen consumption and creatinine excretion .Appl Physiol 1952;(5):73-78

8. Stuphen J.L: Anthropermetric determinant of creatinine excretion in preterm infants.*Paediatrics*1982 ;(69):719-723

9. Vestergaard P., Leverett R., Orangeburg N.Y: Constancy of urinary creatinine excretion.J Lab Clin Med 1958 ;(51):211-18

Section four

Outcome based on the project:

Part Of the research findings were presented at university annual research sessions on10/11/2005 and Annual sessions of Physiological society of Sri Lanka 22/11/2005.

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Comments of the head of the department:

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