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Serum Creatinine, Urea Nitrogen And Endogenous Creatinine Clearance Based Glomerular Filtration Rate In Camels To Evaluate Renal Functions

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Abstract

To evaluate renal functions in camel, the study on profiles like Serum Creatinine (SC), Serum urea nitrogen (SUN), SUN/SC ratio was undertaken in institute's herd of camels (n=250) (*Camelus dromedarius)* of all age groups, both sexes and three breeds and in addition Glomerular Filtration rate (GFR) of 18 male camels of 3 breeds (n=6 each) with 6 individual replicates (Total 108 observations) were determined. The values of SC and SUN/SC ratio differ significantly (P< 0.05) between breeds, while those of SUN did not differ (P>0.05). SC was significantly higher in male (P<0.05), while SUN and SUN/SC ratio was significantly higher (P<0.05) in females. The values of SC and SUN/SC ratio did not differed significantly (P>0.05) in different age groups, while SUN differed significantly (P<0.05) between different age groups. Daily Urine Output (UO) and GFR differed significantly (P<0.05) between Bikaneri and Kachchi breeds, while the difference was statistically non-significant (P>0.05) between Bikaneri and Jaisalmeri and Bikaneri and Kachchi breeds. UO and GFR had highly significant (P<0.01) positive correlation ship(r = 0.634). Urine Creatinine (UC) concentration and Total daily Creatinine excreted (TC) did not differ significantly (P<0.05) between breeds. UO and UC had significant (P<0.05) negative correlation ship (r = -0.234). Coefficient of variation within individual for UO, UC, TC and GFR was relatively more indicating less consistency of these profiles. It was felt that under well-monitored and controlled intake of water, GFR could be used to evaluate renal functions in camels.

Keywords: Creatinine clearance, Azotemia, Glomerular Filtration rate

Introduction

Serum urea nitrogen (SUN) and serum creatinine (SC) concentrations are used in veterinary medicine as an indirect indicator of renal glomerular filtration rate to estimate renal functions. It is recognized that these are relatively insensitive to detect minor kidney damage. It is also recognized that their levels might not proportionately alter with increase in kidney damage. Besides that there might be non-renal, pre renal and post renal causes for azotemic levels of these biochemical in serum. About 3/

4th of renal function must be lost or say glomerular filtration rate (GFR) reduced to 25% of normal before abnormalities in SC and SUN concentration are seen (Miller *et al*., 2004). Compared to these serum profiles, GFR is currently considered the best overall indicator of renal functions (Watson *et al*., 2002). Smith (1951) and Winton (1956) observed that GFR measurements remained constant despite variation of 200-600% in urine flow. It is recognized in human that GFR falls suddenly if acute renal failure occur. When a specific measure of GFR is sought, Creatinine clearance (Ccr) may be performed in the species in which it is valid such as Dog, Cat, Horse (Kaneko *et al*, 1997) and camel (Schmidt-Nielsen *et al.*, 1957). Ccr test evaluates how efficiently the kidneys clear creatinine from the blood. Most of the research on this aspect has been done on human. Analogous studies in animal species are scarce. Due to lack of interspecies data, meaningful comparisons are difficult for veterinary clinicians. This study is an attempt to evaluate efficiency of SC, SUN, SUN/SC ratio and Ccr profiles as an indicator of functioning of kidney in camels.

Materials and Methods

The study on Serum Creatinine (SC), Serum urea nitrogen (SUN) profiles and SUN/SC ratio was conducted on institute's herd of camels (n=250) (*Camelus dromedarius*) of all age groups, both sexes and three breeds (Viz. Bikaneri, Jaisalmeri and Kachchi). Glomerular Filtration rate (GFR) of 18 male camels of 3 breeds (n=6 each) with 6 individual replicates (Total 108 observations) were determined through endogenous Creatinine clearance test. The animals were let loose for grazing during daytime and offered dry fodder in the evening. Adult males were used for GFR study and these were confined at stall for 7 days during the study. They were given dry fodder and taken on water hut once daily for watering.

Sample Collections

Blood was collected through jugular vene-puncture and serum was harvested for biochemical evaluations of SC and SUN. Urine samples from males for GFR were harvested through specially designed non-leaky bags applied permanently around their prepuce and penis. The bags were periodically emptied of the urine through an outlet and urine samples were pooled in separate containers to be preserved at refrigerated temperature for 24 hours, at the end of which, the samples were measured for daily urine output (UO) and urine creatinine (UC) concentration.

Biochemical Analysis

Biochemical analysis of SC and UC were accomplished as per methods described by Robert L. Murray, in which creatinie purified with fuller's earth reacts with picrate ions in a highly alkaline solution to form a red complex measured at 509 nm. 200 µl of serum, diluted urine, standard, or water (reagent blank) was pipetted into labeled tubes. To this 100 µl of tungstate reagent and

100 µl of sulfuric acid reagent were added. Mixed well, 3 minutes at room temperature, centrifuge for 5 minutes at 12,000 g, followed by transfer of 300 µl of the supernatant into a second tube. To this 500 µl of fuller's earth from a vigorously stirred suspension was added. Mixed for 1 minute, centrifuge for 2.5 minutes at 12000g, followed by aspiration of the supernatant fluid completely. Finally 500 µl of picrate solution was added to the pellet.This was mixed vigorously until the pellet was thoroughly re suspended. Let the mixture stand in a 30° C incubator for 30 minutes, centrifuge for 1 minute, and measure the absorbance of the supernatant fluid versus the reagent blank at 509 nm at 30°C. SUN was analyzed by Di acetyl mono oxime method (Natelson, 1957).

GFR: SC, UO and UC were measured for 18 male camels for 6 replicates each. Daily TC and GFR were derived from these biochemical parameters using standard formulations as shown below.

TC = Urine concentration (mg/L) X Urine output (Litres)

GFR = Urine creatinine (mg/L) X Urine volume (ml/minute) ÷ Serum creatinine (mg/L)

Statistical Analysis

Effect of breed, age and sex on SC, SUN and SUN/SC ratio was evaluated through One-way analysis of variance. UO, UC, TC and GFR between breeds were compared by Paired t test. Within individual coefficient of variation was derived for UO, UC, TC and GFR. Pearson's coefficients of correlation were calculated between UO &UC and UO &GFR. Computerized SPSS 10.0 was used for these statistical analyses.

Results and Discussion

Serum Creatinine, Urea Nitrogen and Urea Nitrogen Creatinine Ratio:

Table 1 shows the mean values of SC, SUN and SUN/SC ratio of 3 breeds of camels. The values of SC and SUN/SC ratio differ significantly ($P<0.05$) between breeds, while those of serum urea nitrogen did not differ ($P>0.05$). Table 2 shows sex wise mean values of SC, SUN and SUN/SC ratio. SC was significantly higher in male ($P<0.05$), while SUN and SUN/SC ratio was significantly higher in females. Table 3 shows age wise mean values of SC, SUN and SUN/SC ratio. The values of SC and SUN/SC ratio did not differ significantly ($P>0.05$) in different age groups, while SUN differ significantly ($P<0.05$) between different age groups. The values of SC observed in present study in camel were higher than those of Kataria *et al*. (2003) of 1.357±0.097 in camels. Different methodologies of quantification might have produced differences in results. Methodology used in present study was based on adsorption of SC from protein free filtrate by fuller's earth removing interfering substances is expected to be superior. Our values were also higher than those reported in men (0.8 to 1.3 mg/dl) and women (0.6 to 1.0 mg/dl) by Palmer (2006). These

species differences might be due to wide differences in total muscle mass between camels and human. In blood, Creatinine is produced from phospho-creatine of muscle tissues, which is utilized by the body spontaneously, irreversibly and non-enzymatically for energy requirements of the tissues. This amount is directly proportional to the individual's muscle mass. Therefore, a stable amount of Creatinine is presented to the kidneys daily for excretion. The SC concentration can vary based on a number of factors including animal's diet, muscle mass and gender (Miller *et al*., 2004). Values of SC were significantly higher in male as compared to female, which is in agreement to the previous findings of Latimer *et al*. 2003 that Creatinine levels depend on total muscle mass and males in general being heavier than females are expected to have higher values of SC. Age wise comparison of SC revealed that it did not differ between different age groups. This may be possible as SC level depends on muscle mass and kidney functions. Muscle mass is expected to increase in young and middle age, while it decreases in old age. The greater Creatinine produced from muscle mass may be compensated by strong kidney functions in young and middle aged, and low production in old aged by reduced kidney functions. SUN, like SC, is also a waste product formed through the body's metabolism of protein. The values of SUN observed in present study falls within normal reference range of 7-25 mg per dl in human. The values of SUN did not differ significantly between breeds. It was significantly higher in females as compared to males. It also increased with increasing age. These findings resemble to those reported in human that UN levels increases with age. However, the present study differs from those of human for UN profiles in relation to sex. In human, higher levels are expected for males, whereas in our studies in camel, recorded higher levels in females. It might be difficult to exactly find out differences in SUN levels in relation to sex between present study in camel and those reported previously in human, but one major differences in the two species worth mentioning is urea recycling in the stomach of camel by ruminal flora and fauna is not found in human stomach. The values for UN/C ratio observed also resembles to those of normal values reported for human beings of 10-20.

Urine Output

Urine output (UO) in 24 hours in 18 male camels of 3 breeds monitored for a total of 108 times (6 replicates each camel) has been presented in Table 4. It ranged from 2.596± 0.107 to 4.616±0.403 liters in Bikaneri, 2.386±0.184 to 4.373± 0.228 liters in Jaisalmeri and 2.133±0.237 to 4.165±0.242 in Kachchi. Mean UO in 6 camels each of Bikaneri; Jaisalmeri and Kachchi breed measured 3.763±0.208, 3.166±0.166 and 3.119±0.198 liters, respectively. Paired t test revealed that mean UO differ significantly between Bikaneri and Jaisalmeri ($P<0.05$) and Bikaneri and

Kachchi (P<0.05), but it did not differ significantly (P>0.05) between Jaisalmeri and Kachchi breeds. Within individual coefficient of variation ranged from 9.23-47.72%, 10-36.13% and 12.50-49.10% in 3 breeds, respectively. Inter individual differences between 6 camels each of 3 breeds were non significant (P>0.05) in majority of cases (b=11/15, J=10/15 and K=9/15), while few were statistically significant (P<0.05) (B=4/15, J=5/15 and K=6/15). Yagil (1993) also reported daily UO of 3-5 L in camel, which is very low as compared to cattle of similar weight of 20 L. Siebert and Macfarlane (1971) also reported urine flow rate of 1.0-2.5 ml/ minute in summer in camels, which comes to around 1.440-3.600 L/ day. Daily UO observed in present study was less than those reported by Kataria *et al*. (2003) of 8.971±0.951 ml/minute in camel, which comes to around more than 12 liters a day. Within individual coefficient of variation for daily UO was also great. Factors like water intake (Jaffrey Wallach (1995)), glomerular filtration rate (GFR) and tubular re absorption (Rose, 2007)) determine UO. The normal amount of urine passed every day should be nearly equal to the intake of fluid for that day. Quite probable that variation in daily UO within individual might be due to different water intake on different days. GFR and difference between glomerular filtration rate and tubular re absorption are other important factors, which determine the amount of urine flow. Camel can conserve water by reducing urine flow. A dehydrated camel can have a urine output as low as 0.5-1 L per day (Schmidt-Nielsen *et al*., 1956). Urine flow rate was more (4-5.7 ml/minute) in camel (Siebert and Macfarlane (1971) when it had free access to water and it was reduced to 1-1.6 ml/minute after 1 day without water and it was further reduced with increased dehydration. This is speculated to be due to camel's extra ordinary kidney functions (Yagil, 1993).

Urine Creatinine Concentration

Urine creatinine (UC) in 24 hours in 18 camels of 3 breeds monitored for a total of 108 times (6 times each camel) has been presented in Table 4. It ranged from 1380.08±151.03 to 2224.66±29.181 mg/L in Bikaneri, 1278.3±198.66 to 2198.13±99.08 mg/L in Jaisalmeri and 1669.83±240.81 to 2410±261.82 mg/L in Kachchi. Mean UC in 6 camels each of Bikaneri, Jaisalmeri and Kachchi breed measured 1773.22±75.71, 1757.87±72.00 and 1912.89±81.26 mg/ L, respectively. Within individual coefficient of variation ranged from 2.92-39.78%, 7.73-36.67% and 8.56-32.15% in 3 breeds, respectively. Paired t test revealed that mean UC did not differ significantly between breeds. These values are higher than those of Kataria *et al*. (2003) of 91.338±5.838 mg/dl in camel. But daily UO in later was much greater than during present study. Urine Creatinine concentration is affected by volume of the urine produced, rising as the volume falls and vice versa. A significant negative correlation ship was also observed between UC and UO

in present study. Values observed in present study are also higher than those reported for Holstein Friesian and hybrid cows in Urmia of 156-300 mg/L of urine (Ramin et al., 2005). Here again, higher Daily UO in cows might well be responsible for low urine Creatinine concentration. In steady state conditions the amount of Creatinine in the urine closely reflects Creatinine production in the muscles, because contributions of loss of Creatinine in GIT and tubular secretion in kidney are negligible.

Pearson's Coefficient of Correlation Between Daily UO and UC

Correlation between daily UO and UC has been presented in Table 5, which shows that the two are negatively correlated (r=-o.204) and the correlation is statistically significant (P<0.05)

Total Creatinine Excreted in 24 Hours

Total Creatinine (TC) excreted in 24 hours in 18 camels of 3 breeds monitored for a total of 108 times (6 times each camel) has been presented in Table 4. It ranged from 4.980±0.220 to 9.168±1.156 gm per day in Bikaneri, 3.827±0.193 to 9.596±0.586 gm in Jaisalmeri and 4.387±0.727 to 7.091±0.500 gm in Kachchi. Mean TC excreted per day in 6 camels each of Bikaneri, Jaisalmeri and Kachchi breed for 6 replicates measured 6.394±0.354, 5.532±0.384 and 5.630±0.691gm per day, respectively. Within individual coefficient of variation ranged from 21.18-45.8%, 11.26-37.80% and 16.31-36.96% in 3 breeds, respectively. Paired t test revealed that TC excreted per day did not differ significantly between breeds (P>0.05). These values resemble to those of Kataria *et al*. (2003) of 5.153±0.343 and 7.93±0.4 g/day of creatinine excreted daily in camel's urine in summer and winter seasons, respectively. Within individual coefficient of variation was more. These findings differ from those of Folin, 1905 and Shaffer, 1908 in human that quantity of creatinine excreted by human on a meat free diet is constant, but resemble to others, who reported day to day variation of 25% (Maw, 1947 and Albanese and Wangerin, 1944, ± 20% (Jones (2005) or 15% (James *et al.*, 1988) intra individual variability in urine creatinine in human. Singh *et al*. (1978) also reported low daily consistency within individual and greater variability between individual in creatinine excretion. Creatinine excretion has also been observed to be dependent on body weight. But, body weight alone could not explain satisfactorily wide variability of creatinine excretion. Moreover, Bickhardt and Dungelhoff, 1994 observed creatinine excretion in sheep negatively correlated with body weight. Sex, race and age differences on creatinine excretion have also been observed (James *et al.*, 1988). Creatinine excretion has been more in male as compared to female. Variations on account of obesity/ fatness have also been reported. Mc Clugage (1931) observed that creatinine output was low

in obese persons. Ashworth and Brody (1933) also reported that variation in creatinine coefficient was low in fat animals. Contrarily, Rebhan and Donker (1960) observed lower Creatinine coefficient in fat steers but higher variation in fat bulls than their thin mates.

Glomerular Filtration Rate

Glomerular Filtration Rate (GFR) in18 camels of 3 breeds monitored for a total of 108 times (6 times each camel) are presented in Table 4. It ranged from 167.29±7.00 to 325.83±46.23 ml/minute in Bikaneri, 133.34±10.00 to 323.68±29.71 ml/minute in Jaisalmeri and 141.82±18.76 to 553.49±17.09 ml/minute in Kachchi. Mean GFR in 6 camels each of Bikaneri, Jaisalmeri and Kachchi breed for 6 replicates measured 229.87±13.52, 184.52±14.70 and 190.61±10.67 ml/minute, respectively. Within individual coefficient of variation ranged from 9.34-36.01%, 9.87-47.4% and 14.70-29.5% in 3 breeds, respectively. Correlations between GFR and daily urine output are presented in Table 6, which showed a significant ($P<0.01$) correlation between GFR and UO. Paired t test revealed that GFR did not differ significantly between Bikaneri and Jaisalmeri and Jaisalmeri and Kachchi breeds ($P>0.05$), while it was statistically significant ($P<0.05$) between Bikaneri and Kachchi breeds. GFR of 235±30 (Etzion and Yagil, 1986), 377±3.6 (Siebert and Macfarlane, 1971) ml/minute in camels and 73 and 125 ml/minute in 2 female llamas (Becker *et al*., 1956) have been reported, which resembles to average figures observed in present study. However, Kataria *et al*. (2003) reported higher values of 582.41±14.193 ml/minute in camels. Within individual coefficient of variation ranged from 9.34-36.01%, 9.87-47.4% and 14.70-29.5% in 3 breeds, respectively, which was high and does not corresponds with the views expressed by Smith, 1951 and Winton, 1956 that GFR remains fairly constant despite wide variation of urine flow. In fact, a significant positive correlation ($r = 0.634$) was found between variations in GFR and UO in present study in camel. Variations in GFR due to dehydration, season, diet and species have been observed. Etzion and Yagil (1986), Siebert and Macfarlane (1971), Maloiy (1972), Yagil (1985) and Yagil and Berlyne (1978) have all observed significant decline in GFR due to dehydration in camel. The effect of dehydration on GFR was more pronounced in summer than in spring season (Yagil and Berlyne, 1978 and Yagil, 1985). Anti diuretic and Aldosterone hormones might play important role in determining GFR. Decline in GFR during dehydration was found associated with increased concentration of ADH and aldosterone (Yagil and Etzion, 1979). Upon rapid rehydration the ADH and aldosterone concentration declined within 1 hr in the camel leading to rapid increase in GFR. Dehydration can cause hypovolemia, which leads to reduced renal blood flow and GFR (Kaneko *et al*., 1997). Inter

species differences in GFR have been reportd, as for example cattle and sheep had a higher basic GFR than camel (Siebert and Macfarlane, 1971 and Macfarlane, 1984). Osbaldiston and Moore (1971) reported CCr values of 1.39-1.68 ml/minute/Kg body weight in cattle. Similarly, Zatzman *et al*. (1982) have reported higher GFR of 211±56 ml/ minute/ 100 kg body weight in Pony and Horses. In Men and Women normal GFR of 125±25 and 95±20 ml/minute, respectively have been reported. GFR was found considerably greater in sheep maintained on a high protein diet (Gans and Mercer, 1962). Under endogenous Creatinine clearance method used for GFR determinations as done in present study, erroneous results might arise due to incomplete emptying of bladder prior

Table 1: Serum Creatinine, Urea nitrogen and Urea nitrogen/Creatinine ratio in 3 breeds of camels

Breed	Serum creatinine (mg/dl)	Serum urea nitrogen (mg/dl)	Serum urea nitrogen/ Creatinine ratio
Bikaneri	1.88 ± 0.05[a]	21.51 ±1.10	12.30±0.83[d]
Jaisalmeri	2.19 ± 0.07[b]	22.47 ± 1.45	10.38±0.49[e]
Kachchi	2.03 ± 0.08[c]	19.45 ± 1.00	10.13±0.60[f]

Note: Values with different superscripts in a column differ significantly (P<0.05).

Table 2: Sex wise serum Creatinine, Urea nitrogen and urea nitrogen/Creatinine ratio in camels.

Sex	Serum creatinine (mg/dl)	Serum urea nitrogen (mg/dl)	Serum urea nitrogen /Creatinine ratio
Male	2.26±0.08[a]	18.97±1.61[c]	8.43±0.53[e]
Female	1.94±0.04[b]	22.29±0.76[d]	12.08±0.48[f]

Note: Values with different superscripts in a column differ significantly (P<0.05).

Table 3: Age wise serum Creatinine, urea nitrogen and urea nitrogen/Creatinine ratio in camels.

Age Group	Serum creatinine(mg/dl)	Serum urea nitrogen(mg/dl)	Serum urea nitrogen /Creatinine ratio
< 1 year	2.05±0.08	16.70±1.57	9.38±1.39
1-2 year	1.87±0.09	15.36±1.40	8.32±0.79
2-5 year	2.16±0.13	25.62±2.01	12.26±1.02
5-10 years	2.00±0.07	23.41±1.80	11.86±0.06
Above 10 year	2.03±0.04	21.05±0.80	11.03±0.47

Table 4: Urine output, urine Creatinine, total daily Creatinine excreted, glomerular filtration rate, and Creatinine clearance in 3 breeds of male camels

		Bikaneri	Jaisalmeri	Kachchi
Daily urine output (L)	Minimum individual average	2.59±0.10	2.38±0.18	2.13±0.23
	Maximum individual average	4.61±0.40	4.37±0.22	4.16±0.24
	Overall average	3.76*±0.20	3.16±0.16	3.11±0.19
Urine Creatinine mg/L	Minimum ndividual average	1380.0±151.0	1278.3±198.	1669.8±240.8
	Maximum individual average	2224.6±29.1	2198.1±99.0	2410±261.8
	Overall average	1773.2±75.7	1757.8±72.0	1912.8±81.2
Daily Creatinine excreted (G)	Minimum individual average	4.98±0.22	3.82±0.19	4.38±0.72
	Maximum individual average	9.19±1.15	9.59±0.58	7.09±0.50
	Overall average	6.39±0.35	5.53±0.38	5.63±0.69
Glomerular Filtration rate as determined by Creatinine clearnce ml/min	Minimum individual average	167.2±7.0	133.3±10.0	204.2±27.0
	Maximum individual average	325.8±46.2	323.6±29.7	390.3±25.7
	Overall average	229.8±13.5*	184.5±14.7	190.6±10.6*
Creatinine clearance per kg body weight (ml/min)	Minimum individual average	0.282±0.011	0.222±0.015	0.273±0.036
	Maximum individual average	0.549±0.057	0.602±0.051	0.646±0.063
	Overall average	0.451±0.024	0.257±0.083	0.450±0.026

Table 5: Pearson's coefficient of correlation between daily urine output and urine Creatinine concentration

		Urine Output	Urine Creatinine Concentration
Urine Output	Pearson's correlation	1.000	-0.204*
	Significance (2-tailed)	-	0.034
	Number of observations	108	108
Urine Creatinine Concentration	Pearson's correlation	-0.204*	1.000
	Significance (2-tailed)	0.034	-
	Number of observations	108	108

Correlation is significant at the 0.05 level (2-tailed)

Table 6: Correlations between urine output and Glomerular Filtration rate

	Glomerular Filtration Rate	Urine Output
Glomerular Filtration rate	1.000	0.643**
Significance (2-tailed)	-	.000
N	108	108
Urine Output	0.643**	1.000
Significance (2-tailed)	.000	-
N	108	108

** Correlation is significant at the 0.01 level (2-tailed).

to and after termination of urine collections. But in present study, timed urine collections were continued for 6 days continuously which might have helped in minimizing the error. Looking into these facts, application of GFR to evaluate renal functions is possible only under normo-hydrated and non-azootemic animals (Miller *et al*., 2004). Research on altered GFR, either low or higher than normal and its possible applications in identifying kidney function disorders have been extensively worked out in human. According to them Creatinine clearances lower than normal indicated that one or both kidneys are not working normally. Low Creatinine clearance has been identified with blockage in blood vessels going to the kidneys, diseased or damaged kidneys due to high blood pressure, diabetes, infection and auto immune diseases, Kidney stone, Kink in the ureter, pressed ureter from enlarged prostrate or a tumor. Increase Creatinine clearance rates may occasionally be seen during pregnancy, exercise, diets high in meat, muscle injury, burns, Co poisoning and hypothyroidism. Similar sort of information in animals particularly in camel is scarce. The preliminary attempts in this study may be extended further to understand renal physiology

in camels.

Conclusion

Data on Serum Creatinine, urea nitrogen, serum creatinine/urea nitrogen ratio and glomerular filtration rate in dromedary camel herds were created for future advances in the renal physiology to evaluate renal functions in this species.

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