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Establishing Biochemical Reference Intervals for Semi-Captive Elephants in Tamil Nadu, India

Rengarasu Madeswaran[1], Parvathinathan Sandhya[1], M. Gabriel Paulraj[1]*. Arumugam Udhayan[1], Manikka G. Ganesan[1], Rangasamy Kanchana[2], Arulmani Manimozhi[1] and Dhayanithi Vasanthakumari[1]

[1]*Advanced Institute for Wildlife Conservation (Research, Training & Education), Vandalur, Chennai, Tamil Nadu, India*
[2]*Tamil Nadu Wildlife Crime Control Bureau, Chennai, Tamil Nadu, India*
**Corresponding author's email: gabriel_paulraj@yahoo.com*

Abstract. Serum biochemical parameters were studied in 65 Asian elephants maintained at Anamalai Tiger Reserve, Mudumalai Tiger Reserve, Srivilliputhur Megamalai Tiger Reserve and Elephant Rescue and Rehabilitation Centre in Tamil Nadu, India, from November 2022 to July 2023. Serum was separated from blood and 22 parameters were analysed. In male individuals at Anamalai, the blood urea nitrogen and calcium levels were significantly higher in the age group 1–15 years than >50 years. In Mudumalai, glucose concentration in females of 1–15 years of age was significantly higher than in 26–50 year-old females and >50 year-old females. Glucose, total protein and creatinine levels showed significant variations between the four seasons of the year.

Introduction

The Asian elephant (*Elephas maximus*) is distributed in 13 range countries. From ancient times, elephant capture, training and use for various purposes have been common in Indian states. Elephant camps in India like Theppakadu in Mudumalai Tiger Reserve (MTR) and Kozhikamuthi in Anamalai Tiger Reserve (ATR) were established in Tamil Nadu by the Forest Department. Theppakadu camp was set up in 1910 and Varagaliar camp in Topslip in 1956. The Varagaliar camp was shifted to Kozhikamuthy in 2007 (Baruah 2023).

Camp elephants are taken daily for a morning bath in a nearby river and then given a nutritious meal, at around 9:00 am. Afterwards they are set free to graze in nearby forest areas. They are taken for a bath in the evening, and evening feed is given at around 6:00 pm. During British rule, camp elephants were utilised for logging. Currently, they are used to patrol national parks, kumki operations, drive wild elephants, wild animal capturing operations and tourism. The elephants are semi-free ranging. MTR camps house 27 elephants and ATR camps house 28 elephants. Health monitoring of captive elephants under controlled living conditions is important because they may be prone to microbial and parasitic infections (Punya *et al*. 2021; Gautum & Koju 2022).

Diagnosis using blood and serum plays an important role in the early detection of health problems in elephants (Mikota 2006). Liver enzymes such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin, as well as blood glucose and creatinine are basic analytes to diagnose various diseases and abnormalities such as hyperglycaemia, stress, kidney ailments, hepatic diseases etc. (Kerr 2002; Mikota 2006; Thapa 2010). Serum biochemical tests are also important for the regular health monitoring of camp elephants, as they assess the functioning of organs such as the liver, kidneys and pancreas.

Serum biochemical values of African and Asian elephants have been studied by many investigators (Sreekumar & Nirmalan 1989; Sarmah *et al*. 1999; Wijesekera *et al*. 2008; Allwin *et al*. 2015; Edwards *et al*. 2020). A few serum bio-

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chemical studies have also been conducted in camp elephants in Tamil Nadu (Allwin *et al*. 2015; Veeraselvam *et al*. 2021).

Serum biochemical concentrations maybe affected by many factors such as type of food, exercise, seasonal variations, physiological condition of vital organs, stress, tissue damage and parasitic infection. Therefore, regular serum analysis of camp elephants is necessary for monitoring an individual's health. Perrin *et al*. (2020) have reported on the applicability of population-derived reference intervals of haematological and biochemical parameters for Asian elephants and stated that individual normal values should be used for the interpretation of blood results.

Repeated sampling is essential to establish reliable individual reference intervals for serum biochemical parameters of elephants in specific camps, which is lacking. The present study was undertaken to establish reference intervals for serum biochemical parameters of camp elephants in MTR, ATR and Elephant Rescue and Rehabilitation Centre (ERRC) in Tiruchirappalli and Srivilliputhur Megamalai Tiger Reserve (SMTR).

Materials and methods

Study area and study period

Blood samples were collected from camp elephants at Theppakadu (11°57'91.2"N; 76°58' 40.3"E) and Abhayaranyam (11°55'71.1"N; 76°56'19.5"E) elephant camps in MTR, Kozhikamuthi (10°44'53.8"N; 76°84'88.5"E) and Varakalayar (10°41'92.9"N; 76°86'66.1"E) camps in ATR, ERRC and SMTR.

The study was conducted between November 2022 and July 2023 with permission obtained from the Principal Chief Conservator of Forests and Chief Wildlife Warden, Tamil Nadu (Letter Ref. No. WL5(A)/28609/2022; dated 22-11-2022 and Permission No. 85/2022).

In Tamil Nadu, summer starts in March and ends at the end of May. The southwest monsoon season is between June and September and the Northeast monsoon season is from October to December. The winter season occurs from January to February. Blood samples were collected four times in the months of November 2022 (northeast monsoon), February 2023 (winter), May 2023 (summer) and July 2023 (southwest monsoon).

Elephants were categorised into size classes according to Fernando *et al*. (2022).

Blood sample collection and serum separation

Samples were collected from a total of 65 elephants. In ATR, blood samples were collected from 25 elephants (16 males and 9 females) of which four were sub-adults and 21 were adults. In MTR, blood was collected from 24 elephants (18 males and 6 females) which included one juvenile-II, one juvenile-III, two sub-adults and 20 adult individuals. At ERRC 10 adult elephants were accessed for blood samples. At SMTR, 6 elephants (one sub-adult and five adults) were accessed for blood sample collection. The rescued elephants in ERRC and SMTR were all females and were between the ages of 17–70 years.

From each elephant, 10 ml of blood was collected by the Forest Veterinary Assistant Surgeon (FVAS) from the auricular vein in the posterior part of the ear. The area surrounding the vein was cleansed with 70% alcohol and blood was drawn using a butterfly needle. The collected blood was divided into two aliquots of 5 ml each. One aliquot was collected in a clot activator tube and allowed to clot to separate serum. The serum was separated by centrifugation at 3000 rpm for 10 minutes and immediately stored in an icebox.

Serum parameters

The cold-stored serum samples were transported to the Centre for Animal Care Sciences, Advanced Institute for Wildlife Conservation (AIWC), Vandalur. Serum biochemical parameters measured using the IDEXX Catalyst One (IDEXX Laboratories, Inc., USA) were glucose, creatinine, blood urea nitrogen (BUN), BUN/creatinine ratio, phosphate, calcium, total protein, albumin, globulin, albumin/globulin, alanine aminotransferase (ALT), alkaline phos-

phatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, cholesterol, amylase, lipase, sodium, potassium, sodium/potassium, chloride and osmolality. IDEXX Chem 17 clips and Lyte 4 clips were used to quantify the serum biochemicals. All serum biochemical data were recorded and tabulated.

Reference intervals for serum biochemicals were established from the data. Very different biochemical values observed in any individual (outliers) were identified by GraphPad Prism software and removed.

Statistical Analysis

The mean biochemical values of four samplings taken from each individual and their standard deviations were calculated. Statistical comparisons of serum biochemical analyses between males and females, between four different age groups (1–15, 16–25, 26–50, >50 years) and between four different seasons (summer, southwest monsoon, northeast monsoon and winter) were performed using one-way analysis of variance (ANOVA) and post hoc 'Tukey's test' with the help of GraphPad Prism software. Simple linear regression analysis was done to determine the association between age and some important serum parameters in male and female elephants.

Results

Variations between male and female elephants

The average serum biochemical values of all individuals at ATR, MTR, ERRC and SMTR camps are presented in Table 1. Glucose concentration was significantly higher ($p < 0.05$) in rescued females kept in ERRC and SMTR camps. In ATR, average serum glucose concentration was higher in males than females, but the difference was not significant ($p > 0.05$). A gender-based variation in ALP concentration was observed in ATR and MTR camp elephants, but it was not statistically significant ($p > 0.05$) (Table 1 and Fig. 1).

Serum glucose concentration ranged from a low of 36 mg/dl in a 27-year-old male elephant to a high of 105 mg/dl in a 74-year-old female elephant. Rescued private elephants at ERRC and SMTR camps showed great variations in the serum glucose concentration among individuals. In rescued elephants, glucose concentrations ranged from 40 to 210 mg/dl. Eleven rescued elephants showed >130 mg/dl glucose concentrations. Cholesterol concentration was significantly higher ($p < 0.05$) in MTR males than in MTR females or elephants at other camps (Table 1).

Figure 1 shows the glucose, ALT and ALP concentrations in ATR and MTR camp elephants (rescued elephants were omitted). The glucose concentration was significantly ($p < 0.05$) lower in females of ATR compared to females of MTR. There were no significant differences in the concentrations of creatinine, blood urea nitrogen, albumin and globulin between male and female elephants at ATR and MTR (Fig. 1).

Significant differences in some serum parameters were correlated with age of ATR camp elephants. In male elephants, blood urea nitrogen concentrations were significantly different ($P < 0.01$) between the age groups "1–15 years and >50 years (Table 2). When analysing calcium concentration in females, the age groups 16–25 and >50 years showed a significant difference ($P < 0.05$). In male elephants, the calcium concentration was significantly different when comparing >50 years old elephants with 1–15 ($P < 0.01$), 16–25 ($P < 0.01$) and 26–50 ($P < 0.05$) years age groups.

The serum biochemical parameters of MTR elephants are presented in Table 1 and Figure 1.

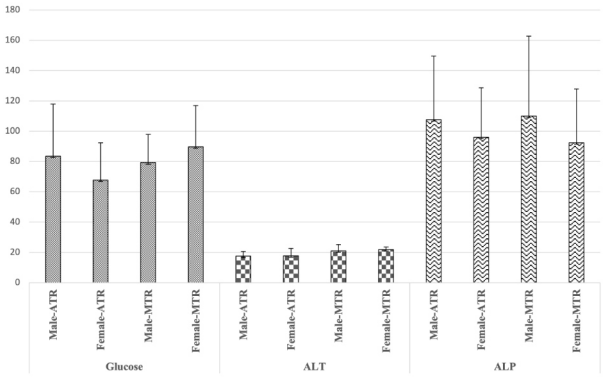

Figure 1. Average glucose (mg/dl), ALT (U/l) and ALP (U/l) concentrations in males and females at ATR and MTR camps. Each bar represents mean ± SD of three sample collections.

Table 1. Average serum biochemical values of male and female elephants at ATR and MTR camps and rescued private elephants at ERRC and SMTR (mean ± SD). If the mean values in a row are significantly different ($p < 0.05$, Tukey HSD Post hoc test), they are marked with different letters (a or b).

Parameter	Unit	ATR		MTR		ERRC & SMTR
		Male (N = 29)	Female (N = 24)	Male (N = 53)	Female (N = 22)	Female (N = 24)
Glucose	mg/dl	83.56 ± 34.38[b]	67.87 ± 24.49[b]	79.34 ± 18.57[b]	89.70 ± 29.81[b]	145.18 ± 60.06[a]
Creatinine	mg/dl	1.56 ± 0.58	1.42 ± 0.26	1.31 ± 0.34	1.36 ± 0.09	1.55 ± 0.37
BUN	mg/dl	9.35 ± 2.98	9.86 ± 2.65	8.38 ± 3.03	9.11 ± 1.47	8.68 ± 2.08
BUN/Creatinine		6.79 ± 3.12	7.65 ± 3.09	8.08 ± 6.01	6.98 ± 1.24	5.81 ± 1.79
Phosphate	mg/dl	6.37 ± 1.80[a]	6.72 ± 0.67[a]	4.64 ± 0.62[b]	5.02 ± 0.68[b]	4.68 ± 0.61[b]
Calcium	mg/dl	9.92 ± 0.45	10.16 ± 0.34	9.86 ± 0.48	10.50 ± 0.59	10.1 ± 0.61
Total Protein	g/dl	8.62 ± 1.05	8.80 ± 0.93	8.62 ± 0.55	9.10 ± 1.26	8.82 ± 0.55
Albumin	g/dl	2.70 ± 0.36	2.76 ± 0.29	2.49 ± 0.22	2.93 ± 0.43	3.07 ± 0.41
Globulin	g/dl	5.89 ± 0.92	6.05 ± 0.85	6.12 ± 0.58	6.16 ± 0.86	5.74 ± 0.35
Albumin/Globulin		0.46 ± 0.08	0.46 ± 0.08	0.40 ± 0.06	0.47 ± 0.04	0.53 ± 0.09
ALT	U/l	17.60 ± 3.00	17.80 ± 5.06	21.05 ± 4.10	21.85 ± 1.88	19.00 ± 7.44
ALP	U/l	107.58 ± 42.03	96.03 ± 32.59	109.98 ± 52.82	92.44 ± 38.76	139.12 ± 49.17
GGT	U/l	1.41 ± 4.05	0.81 ± 2.44	3.75 ± 8.05	0.00 ± 0.00	0.31 ± 0.70
Total Bilirubin	mg/dl	0.29 ± 0.11	0.26 ± 0.13	0.51 ± 1.12	0.33 ± 0.14	0.21 ± 0.10
Cholesterol	mg/dl	44.91 ± 11.10[b]	45.70 ± 13.47[b]	59.28 ± 12.14[a]	43.34 ± 9.80[b]	43.62 ± 9.64[b]
Amylase	U/l	1597.07 ± 210.71[b]	1421.31 ± 534.15[b]	1762.00 ± 214.70[a]	1740.04 ± 261.95[a]	1711.87 ± 93.81[a]
Lipase	U/l	23.86 ± 15.93[b]	36.31 ± 12.81[a]	43.05 ± 15.62[a]	40.77 ± 7.28[a]	34.54 ± 17.55[ab]
Sodium (Na)	mmol/l	128.45 ± 1.71	131.01 ± 1.21	130.08 ± 2.21	130.83 ± 1.62	130.12 ± 2.63
Potassium (K)	mmol/l	5.93 ± 0.95	5.87 ± 0.46	4.94 ± 0.23	4.75 ± 0.26	5.87 ± 0.77
Na/K		22.61 ± 3.18	23.38 ± 1.53	26.43 ± 1.48	27.69 ± 1.96	22.56 ± 2.87
Chloride	mmol/l	93.85 ± 1.36	94.74 ± 1.53	94.38 ± 1.80	94.01 ± 2.00	95.06 ± 2.34
Osmolality	mmol/kg	257.75 ± 3.80[a]	261.16 ± 2.13[a]	258.20 ± 4.70[a]	260.44 ± 2.54[a]	179.75 ± 5.54[b]

Serum glucose concentration was lower in male elephants than in females. But the difference was not significant. The highest blood glucose concentration was recorded in a 3-year-old female calf, and the lowest in a 37-year-old male. The average creatinine concentration was the same in both male and female elephants. The average cholesterol concentration was significantly higher ($p < 0.05$) in male elephants than in females at MTR (Table 1).

The individual's age was found to affect some serum biochemical parameters in both sexes. Glucose concentration in females at MTR showed significant differences between the age groups 1–15 years and 26–50 years ($P < 0.01$) and 1–15 years vs. >50 years ($P < 0.01$) (Table 2). The creatinine concentration in males at MTR also differed by age. The creatinine concentration of the age group 16–25 years was significantly different from the 1–15 ($P < 0.01$),

Table 2. Serum glucose, creatinine and BUN in different age groups at ATR and MTR camps. Values in a row carrying different letters (a, b or c) are significantly different (p < 0.05, Tukey HSD Post hoc test).

Parameter	Sex	Camp	1–15 years		16–25 years		26–50 years		>50 years	
			Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Glucose	Male	ATR	70.22 ± 27.65	7	76.94 ± 22.79	7	86.76 ± 33.08	10	102.33 ± 77.31	5
		MTR	98.83 ± 6.62^{a}	10	70.41 ± 14.02^{b}	5	76.81 ± 24.21^{b}	14	75.85 ± 16.05^{b}	25
	Female	ATR	34.0	1	68.27 ± 21.07	8	83.0 ± 1.4	3	72.24 ± 28.62	12
		MTR	133.75 ± 0.00^{a}	4	114.0 ± 0.00^{a}	3	70.0 ± 6.0^{b}	7	75.25 ± 25.45^{b}	8
Creatinine	Male	ATR	1.17 ± 0.19	7	1.85 ± 0.65	7	1.58 ± 0.48	10	1.6 ± 1.27	5
		MTR	1.13 ± 0.08^{b}	10	1.81 ± 0.12^{a}	5	1.34 ± 0.28^{b}	14	1.22 ± 0.38^{b}	25
	Female	ATR	1.9	1	1.19 ± 0.11	8	1.53 ± 0.00	3	1.43 ± 0.20	12
		MTR	1.27 ± 0.00^{b}	4	1.46 ± 0.00^{a}	3	1.31 ± 0.07^{ab}	7	1.42 ± 0.07^{a}	8
BUN	Male	ATR	11.61 ± 0.97^{a}	7	8.83 ± 3.32^{a}	7	10.04 ± 2.24^{a}	10	4.33 ± 0.94^{b}	5
		MTR	11.0 ± 2.00^{a}	10	8.91 ± 0.82^{ab}	5	9.06 ± 3.03^{ab}	14	6.85 ± 3.14^{b}	25
	Female	ATR	7.0	1	11.38 ± 1.91	8	10.0 ± 0.00	3	9.41 ± 3.32	12
		MTR	7.5 ± 0.00^{c}	4	8.33 ± 0.00^{bc}	3	10.91 ± 0.58^{a}	7	8.5 ± 0.35^{b}	8

Table 3. Serum glucose, creatinine and BUN concentrations at different seasons in male and female elephants at ATR and MTR camps. Values in a row carrying different letters (a, b or c) are significantly different (p < 0.05, Tukey HSD Post hoc test). NA = not available.

Parameter	Sex	Camp	November 2022		February 2023		May 2023		July 2023	
			Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Glucose	Male	ATR	97.85 ± 16.80^{a}	14	70.42 ± 37.06^{b}	7	24.0 ± 8.83^{c}	8	NA	
		MTR	79.66 ± 34.45	12	79.69 ± 12.76	13	75.07 ± 24.65	14	85.46 ± 35.08	15
	Female	ATR	115.75 ±55.42^{a}	8	61.12 ± 27.04^{b}	8	19.83 ± 8.23^{b}	8	NA	
		MTR	95.16 ±52.97	6	82.00 ± 25.52	5	94.50 ± 31.95	6	85.40 ± 28.07	5
Creatinine	Male	ATR	1.48 ± 0.56	14	1.27 ± 0.41	7	1.81 ± 0.69	8	NA	
		MTR	1.27 ± 0.48	12	1.30 ± 0.45	13	1.35 ± 0.30	14	1.32 ± 0.31	15
	Female	ATR	1.30 ± 0.36	8	1.3 ± 0.19	8	1.53 ± 0.26	8	NA	
		MTR	1.25 ± 0.13^{a}	6	1.24 ± 0.15^{a}	5	1.63 ± 0.25^{b}	6	1.32 ± 0.17^{ab}	5
BUN	Male	ATR	9.28 ± 3.40	14	7.28 ± 3.81	7	9.12 ± 3.94	8	NA	
		MTR	8.58 ± 3.77	12	7.72 ± 4.40	13	8.28 ± 3.33	14	8.13 ± 2.77	15
	Female	ATR	10.62 ± 4.20	8	8.87 ± 1.64	8	10.87 ± 3.09	8	NA	
		MTR	10.50 ± 2.07^{a}	6	8.80 ± 1.78^{a}	5	7.33 ± 2.16^{b}	6	9.60 ± 1.14^{a}	5

26–50 (P < 0.05) and >50 (P < 0.01) year groups. Blood urea nitrogen analysis in females showed a significant difference between the age groups 1–15 and 26–50 years (P < 0.01).

While examining total protein, there was a significant difference between the 26–50 and >50 years (P < 0.01) male age groups at MTR. In females, there existed a significant difference in total protein concentration between the 1–15 and 26–50 years groups (P < 0.01), the 1–15 and >50 years groups (P < 0.01) and the 26-50 and >50 years groups (P < 0.01).

Seasonal impact

Males at ATR showed a significant difference in glucose concentration between November 2022 (northeast monsoon) and May 2023 (summer) (P < 0.01) and between February 2023 (winter) and May 2023 (summer) (P < 0.05) (Table 3). In females, a significant difference was observed between November and February (P < 0.05) and November and May (P < 0.01). Total protein concentrations in males exhibited a significant difference between November and May (P < 0.05).

In MTR it was seen that creatinine concentrations significantly differed between November and May) ($P < 0.05$) as well as between February and May ($P < 0.05$) in females (Table 3). The total protein concentration in males showed a significant difference between May and July (southwest monsoon) ($P < 0.01$).

Figures 2 and 3 show the linear models for understanding the association between age and serum biochemical parameters in male and females at ATR and MTR camps. In ATR, a positive correlation was observed between age and serum glucose, creatinine and cholesterol in both sexes. But the correlations were not significant ($p > 0.05$). A strong negative association between age and BUN ($p < 0.01$) was observed in MTR males. Table 4 provides a reference interval for all serum biochemicals.

Discussion

The study indicated that some serum parameters, such as creatinine, glucose and calcium differed by age in both sexes. Ratnasooriya *et al*. (1999) reported that the glucose level in the serum of Sri Lankan elephants was not significantly affected by age. However, they found that glucose concentration was significantly higher in males (88–186.2 mg/dl) than females

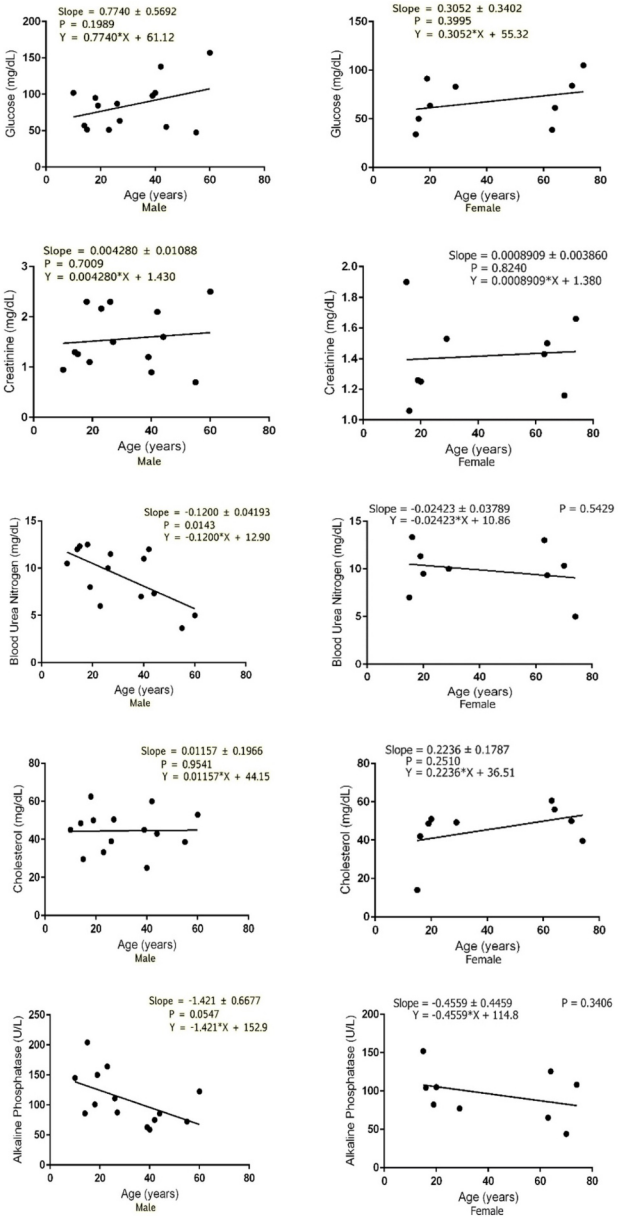

Figure 2. Simple linear regression model to show the association between age and serum biochemicals at ATR camps.

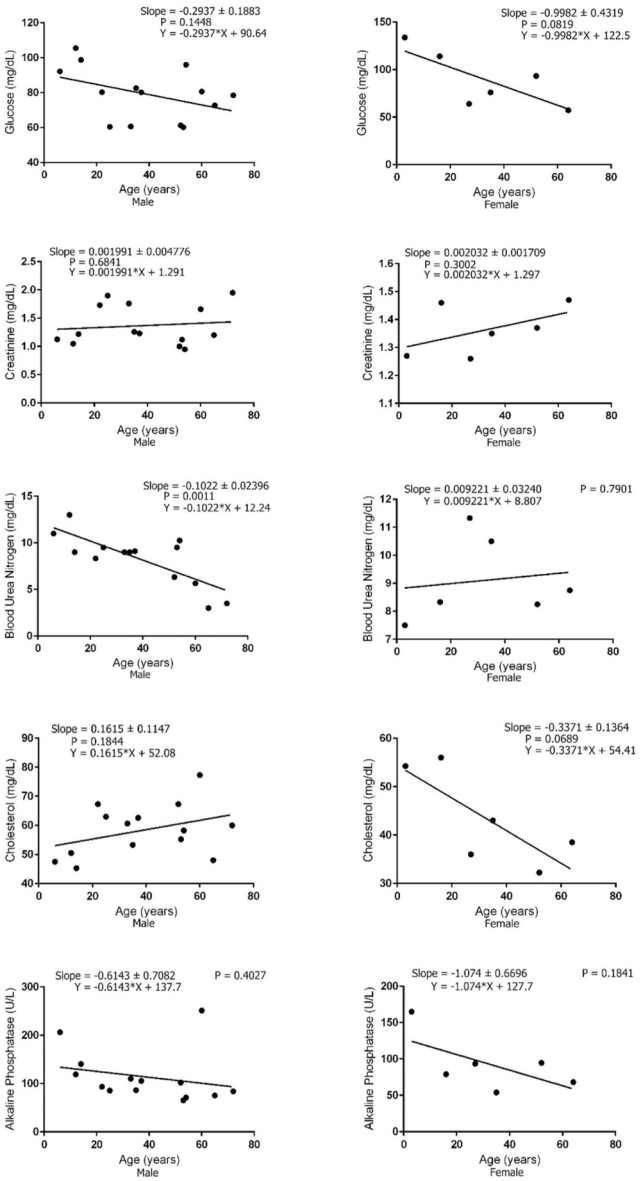

Figure 3. Simple linear regression model to show the association between age and serum biochemicals at MTR camps.

Table 4. Reference intervals for serum parameters of camp elephants based on the values obtained from ATR and MTR camp elephants (N=129).

Parameters	Units	Range
Glucose	mg/dl	67.87 – 89.71
Creatinine	mg/dl	1.31 – 1.56
BUN	mg/dl	8.38 – 9.87
BUN/Creatinine		6.78 – 8.08
Phosphate	mg/dl	4.65 – 6.72
Calcium	mg/dl	9.86 – 10.50
Total Protein	g/dl	8.62 – 9.10
Albumin	g/dl	2.49 – 2.93
Globulin	g/dl	5.89 – 6.17
Albumin/Globulin		0.40 – 0.47
ALT	U/l	17.60 – 21.86
ALP	U/l	92.44 – 109.98
GGT	U/l	0 – 3.75
Total Bilirubin	mg/dl	0.26 – 0.51
Cholesterol	mg/dl	43.34 – 59.28
Amylase	U/l	1421.31 – 1762
Lipase	U/l	23.86 – 43.40
Na	mmol/l	128.45 – 131.01
K	mmol/l	4.76 – 5.93
Na/K		22.61 – 27.69
Chloride	mmol/l	93.85 – 94.74
Osmolality	mmol/kg	257.75 – 261.16

(64.2–128.7 mg/dl). In our study, we observed significantly higher glucose concentrations only in males at ATR. However, in both ATR and MTR camp elephants, the serum glucose was within the reference range that was established by previous investigators (Ratnasooriya *et al*. 1999; Mikota 2006). Silva & Kuruwita (1993) reported that there were no significant differences in the serum biochemical values between males and females of captive elephants in Sri Lanka. In ERRC and SMTR, the rescued elephants showed glucose concentrations beyond 160 mg/dl and up to 210 mg/dl, which were very high compared to the average values reported by Ratnasooriya *et al*. (1999) (mean: 97.55 mg/dl in females) and Allwin *et al*. (2015) (mean: 72 mg/dl).

Biochemical parameters were prone to variation across age groups, but consistent between different samplings from the same individual. This finding corroborates the importance of establishing individual reference ranges for elephants under human care as previously recommended by Perrin *et al*. (2020). Glucose values, in spite of having a high level of variation, were similar across both camps. Creatinine and blood urea nitrogen also stayed within limits previously reported, indicating good renal health. The occurrence of biochemical values within normal limits suggested the absence of organ damage in the sampled camp elephants.

Veeraselvam *et al*. (2021) studied the serum biochemistry in 46 captive elephants in Tamil Nadu. They reported that the parameters were within the normal ranges and found that age-related differences in the parameters were not significant. Allwin *et al*. (2015) collected blood samples only once from 68 captive elephants at forest camps, in temples, circuses and in biological parks and analysed the serum biochemicals. Their findings clearly showed that there were no significant differences in the parameters between males and females. In the present study, blood samples were collected four times in the year corresponding to the four seasons, which helped to arrive at more reliable and precise results. Despite some variation in different seasons, age groups and genders, the results remained replicable and reliable. In MTR, a 60-year-old elephant showed very high ALP and GGT concentrations. Such outliers were excluded while establishing the reference interval for serum biochemical values.

Diet plays an important role in serum glucose levels. In forest camps, all elephants are given cut fodder and a prepared diet consisting of horse gram, ragi, salt and jaggery. We did not consider the effect of diet on serum biochemical parameters. But we recommend such a study. Camp elephants have the benefit of getting immediate diagnosis for any ailments they suffer. These findings can help the forest veterinarians to diagnose deviations from normal hence provide early treatment.

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