Tamil Nadu Forest Department
ADVANCED INSTITUTE FOR WILDLIFE CONSERVATION
(Research, Training & Education)
Vandalur

Project Completion Report
on

'SURVEILLANCE OF CHRONIC KIDNEY DISEASE BY ANALYSIS OF SERUM SYMMETRICAL DIMETHYL ARGININE (SDMA) IN CAPTIVE TIGERS OF AAZP'

Annual Plan of Operations
(APO) Project
2021-2022

August 2023

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CENTRE FOR ANIMAL CARE SCIENCES
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PROJECT COMPLETION REPORT

Title of the Project:	**'SURVEILLANCE OF CHRONIC KIDNEY DISEASE BY ANALYSIS OF SERUM SYMMETRICAL DIMETHYL ARGININE (SDMA) IN CAPTIVE TIGERS OF AAZP'**
Project Category:	Annual Plan of Operations (APO) 2021-22
Project Period:	One Year (August 8, 2022 – August 07, 2023)
Funded by:	Tamil Nadu Government
Implementing Institute and Centre	Centre for Animal Care Sciences, Advanced Institute for Wildlife Conservation, Vandalur – 600 048.
Name of the Project Scientist:	Dr M. Gabriel Paulraj
Name of the Project Assistant:	Ms P. Sandhya

ABSTRACT

Tigers are globally one of the most endangered species as per the IUCN Red List. Of the nine *Panthera tigris* subspecies, three are extinct. Only six subspecies remain today: *P. t. tigris, P. t.altaica, P. t. sumatrae, P. t. corbetti, P. t. Jacksoni* and *P. t. amoyensis*. Tigers exist in eleven Asian countries, with viable populations existing in eight countries including India. Royal Bengal tigers face a high risk of chronic kidney disease (CKD), causing considerable mortality and morbidity. CKD is challenging to detect, as the standard kidney disease markers – creatinine and blood urea nitrogen - only rises significantly after 70% kidney loss. Symmetric dimethylarginine (SDMA) is a more sensitive kidney biomarker, more accurately reflecting glomerular filtration rate and remains unaffected by various factors. Detecting CKD early is crucial for treating captive non-domestic felids in zoos, especially as CKD occurrence rises with age in captivity due to their extended lifespan. In the present study, blood and serum samples from nine adult Royal Bengal tigers housed at Arignar Anna Zoological Park (AAZP) were collected and analysed. Results showed that all the nine tigers are healthy and did not suffer from any renal issues as evidenced by the SDMA levels, which remained under the normal reference range of 0-14 ug/dL, as provided by IDEXX. The SDMA values for the samples collected in this study ranged from 6-11 ug/dL. Certain tigers exhibited age-related issues but they were all seen to be healthy when compared with other serum biochemical and haematological parameters. Hence, it was concluded that SDMA also performed as a good renal biomarker like creatinine and blood urea nitrogen and that all the tigers sampled at AAZP are under good care and exhibit sound health.

INTRODUCTION

Globally tigers are listed as Endangered on the International Union for the Conservation of Nature (IUCN) Red list of Threatened Species. There are nine sub-species under the species *Panthera tigris*, among which *P. t. virgata, P. t. balica* and *P. t. sondaica* have already gone extinct. Till date only *P. t. tigris, P. t. altaica, P. t. sumatrae, P. t. corbetti, P. t. jacksoni* and *P. t. Amoyensis* exist in the whole world. Data on CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) reveals that among 48 countries in Asia, tigers exist only in 11 countries but firm breeding populations exist only in 8 countries. By comparing historic and present range, tigers no longer live in 96 percent of their historic range and unfortunately, much of this decline has occurred in the past decade. In India according to the latest report of Central Zoo Authority, India, approximately there are 389 Bengal tigers in captivity. In addition, there are 3167 tigers in wild range, which is technically reported by All India tiger estimation, 2022that is conducted every four years by the Ministry of Environment,
India.

In India Royal Bengal tigers (*Panthera tigris tigris*) are one of the most important non-domestic felids that are highly prone to chronic kidney disease (CKD).With high incidence reports, significant cause of mortality and morbidity in these felids is due to CKD. Blood creatinine is considered as the gold standard biomarker for kidney disease. But it is tremendously affected by muscle mass and only when 70% of the kidney is lost there is a significant raise in creatinine in the blood. Symmetric dimethylarginine (SDMA) is renal biomarker which is primarily eliminated by kidney through renal clearance. Serum SDMA is highly affected by Glomerular filtration rate (GFR). It is suggested to be a sensitive biomarker for kidney function since it is least affected by extrarenal factors, body mass, age, diet, catabolism or disease rate (Hall *et al*., 2014). Elevation in the level of serum SDMA is significantly noted even with 40% decline in kidney function. SDMA is a most stable molecule that originates from post translational methylation of arginine residues of intranuclear cellular metabolism. Early detection of CKD is immensely important in treating captive non-domestic felids in zoological parks. Occurrence of CKD increases with the age of captive felids (Bartlett *et al.,* 2010; Lawson, 2015). Usually in comparison with wild animals, captive animals have longer life expectancy thus they are highly prone to age-related diseases (Longley, 2011; Tidiere, 2016).

Figure 1. 'Vijayan' – The Royal Bengal Tiger in Arignar Anna Zoological Park

In zoos felids, congenital diseases affecting the kidneys are rare but woefully inbreeding was a major factor indicated for the early onset of renal dysfunction miserably noted in 5 out of nine cubs across 3 litters born to a consanguineous pair of Siberian tigers at San Diego Zoo. And the cause of death is largely associated with glomerulonephritis, metastatic calcification and uraemia. Biochemical and haematological studies are crucial tool for health evaluation and helpful in interpreting the status of physiological functions of various organs. Viral, bacterial and parasitic infections may affect the biochemical parameters (Shrivastav and Singh, 2012). Health monitoring, management, disease diagnosis and treatment should be mandatory for the conservation of captive tigers as it is a key stone species and important member in forest ecology.

Present study was conducted to evaluate the kidney health in captive tigers of AAZP by studying the SDMA levels in serum and another important aim of this study was to establish a reference interval for serum SDMA for captive Bengal Tigers.

1) Estimation of serum SDMA in captive tigers of AAZP
2) Institution of renal protective measures in tigers that are at risk of risk kidney disease
3) To establish standard reference values for the captive tigers of AAZP

Review and Status of Research and Development in the subject

International Status

Chronic kidney disease is a common cause of morbidity and mortality in captive tigers but the sensitivity of creatinine and blood urea nitrogen is highly variable. Estimation of SDMA levels in animals to assess renal issues is gaining traction along other biomarkers like creatinine and blood urea nitrogen as it less affected by extrarenal factors. But research work is very scanty on SDMA analysis in large felids, let alone tigers. Mota *et al*. (2021) studied captive tigers and found out that SDMA is positively associated with creatinine concentration and also assessed that that age and subspecies influenced creatinine, but not SDMA. Youn *et al*. (2017) studied captive lions and Bengal tigers and summarised that SDMA and blood urea nitrogen had higher correlation than SDMA and creatinine. They also found that SDMA, creatinine and blood urea nitrogen concentrations increased with age in tigers and lions. Lamglait *et al*. (2017) studied captive cheetahs and summarised that SDMA levels rose earlier than creatinine and urea, highlighting its ability as an early renal biomarker. They tested on serum samples collected from cheetahs that died due to chronic kidney disease and estimated SDMA levels to range from 14-67 ug/dl. Studies on SDMA analysis in cats are comparatively more prevalent. On studying cats suffering from chronic kidney disease, Hall *et al*. (2014) found that serum SDMA concentrations significantly correlated to glomerular filtration rate and increased earlier serum creatinine.

SDMA had higher sensitivity compared with creatinine. Brans *et al.* (2021) reported that SDMA level of less than 14 µg/dL can be accepted as a safe value in cats (Brans *et al*., 2021) Lanhgorn *et al*. (2017) on studying cats with hypertrophic cardiomyopathy and diabetes mellitus estimated that healthy cats with no medical issues showed a SDMA level of 15 ug/dl. The review of literature shows that SDMA is a promising biomarker for renal health when compared to the traditional likes of creatinine and blood urea nitrogen but there exists a lacuna in its research, especially in captive felids which are under major threat of suffering chronic kidney disease.

National Status

In India Royal Bengal tiger (*Panthera tigris tigris*) is the most important big cat and flagship species in Indian forests. Tigers are highly prone to chronic kidney disease (CKD). Several incidences of CKD, significant mortalities and morbidity due to CKD have been reported worldwide. In Delhi Zoo, seven Bengal tigers, which were the part of conservation, died and among at least four died because of renal dysfunction with high incidence of phosphorous and creatinine.

Cre, BUN, electrolytes, cystatin C and β-Trace Protein are considered as essential biomarkers for kidney health. Creatinine level in urine and serum is often used for the estimation of glomerular filtration rate (Gowda *et al.,* 2010). Urea is the final end product of proteins and amino acids, generated in the liver though urea cycle. In free ranging tigers the BUN range was recorded as 6.5 to 48.2 mg /dl with a mean of 27.9mg /dl (Shrivastav and Singh, 2012). Elevation of BUN is usually associated with renal disease, congestive heart failure, shock, hypertension etc. (Shrivastav *et al.,* 2011).SDMA level elevates earlier than creatinine in the case of CKD. As per previous reports, SDMA increases with 40% loss of kidney health or even as early as 25% loss. Comparatively creatinine will not elevate that much at early stages of complication. There is a significant raise in the level of creatinine only after 75% loss in kidney function. Blood urea nitrogen is also a late marker which is influenced by high protein meal and gastrointestinal bleeding.

SDMA is a promising renal biomarker. Blood Urea Nitrogen (BUN) is also considered as an important biochemical to know the renal function (Shrivastav and Singh, 2012). Shrivastav and Singh (2012) conducted a study on haematological and serum biochemical analysis in Bengal Tigers. In that study SDMA was not included, but other renal markers like creatinine and blood urea nitrogen were included. They reported a mean creatinine value of 2.9 mg/dL with a range of 1.6 to 4.6 mg/dL. Very high range blood urea nitrogen (BUN) of 6.5 to 48.2 mg/dL was reported due to the adlib intake of meat.

The review of literature clearly showed that studies on SDMA in captive tigers are very limited in India. Hence there is a need for undertaking such studies in large population of captive Bengal tigers to understand the normal SDMA level at captive conditions. This study will result in fruitful findings to establish concrete reference intervals.

Materials and Methods

Place of the study: The blood was collected from the Royal Bengal tigers that are housed in Arignar Anna Zoological Park (AAZP) (Fig.2), which is situated in Vandalur in Tamil Nadu.

Figure 2. Arignar Anna Zoological Park (AAZP) at Vandalur, Tamil Nadu

Blood collection: Necessary permission was obtained from Principal Chief Conservator of Forests and Chief Wildlife Warden, Tamil Nadu for blood sample collection from tigers of AAZP. From each tiger, 10 ml of blood was collected for the studies. The blood was collected by Veterinary assistant surgeon from the common tail vein (Dorsal coccygeal vein) or saphenous vein using disposable syringes (Fig. 3). Collected blood was divided into two parts of 5 ml each. One part of blood was directly collected in EDTA tubes and gently mixed. Remaining blood was collected in clot activator tube and allowed to clot for separation of serum by centrifugation at 3000 rpm at 10 minutes. The blood samples were collected from nine tigers.

Figure 3. Veterinarian collecting blood sample from an anesthetized tiger

Serum parameters: The cold-stored serum samples were transpo rted to the Centre for Animal Care Sciences, AIWC, Vandalur and serum parameters were quantified by the instrument IDEXX Catalyst One. The SDMA level was estimated along with other serum biochemical parameters like: glucose, creatinine, blood urea nitrogen, blood urea nitrogen/creatinine, phosphate, calcium, total protein, albumin, globulin, albumin/globulin, alanine amino transferase, alkaline phosphatise, gamma glutamyl transferase, total bilirubin, cholesterol, amylase, lipase, sodium, potassium, sodium/potassium, chloride and osmolality. All serum biochemical data were recorded and tabulated.

Figure 4. Automated Serum Biochemistry analyzer (IDEXX Catalyst One)

Haematological studies: Haematological analysis was done using 'Mindray' (Model: BC-2800 vet.). WBC, lymphocytes, monocytes, granulocytes, lymphocytes (%), monocytes (%), granulocytes (%), RBC, haemoglobin, HCT, MCV, MCH, MCHC, RDW, platelets, MPV, PDW and PCT were quantified and recorded. Three replications of each blood sample were used to study the haematological parameters.

Figure 5. Automated Haematology analyzer ('Mindray': Model: BC-2800 vet.)

Statistical Analysis: Mean values were calculated from replication values. Single linear regression model was used to find out the association between age and some haematological and serum biochemical values using GraphPad Prism software.

Results

The main objective of the present study was to analyse the SDMA level in the serum of captive tigers at AAZP. However, we have studied all possible haematological and serum biochemical parameters besides SDMA in all nine tigers studied (Rithvik, Nakulan, Deva, Athreyan, Vijayan, Meera, Sunitha, Narmatha and Anu). Blood samples were collected only once from all tigers except 'Nakulan', which was collected thrice.

Serum SDMA level: The SDMA levels of these nine tigers were within the reference internal provided by **IDEXX (0-14 µg/dL)**. The range for SDMA calculated from the samples lies between **6-11** µg/dL (Fig. 6).

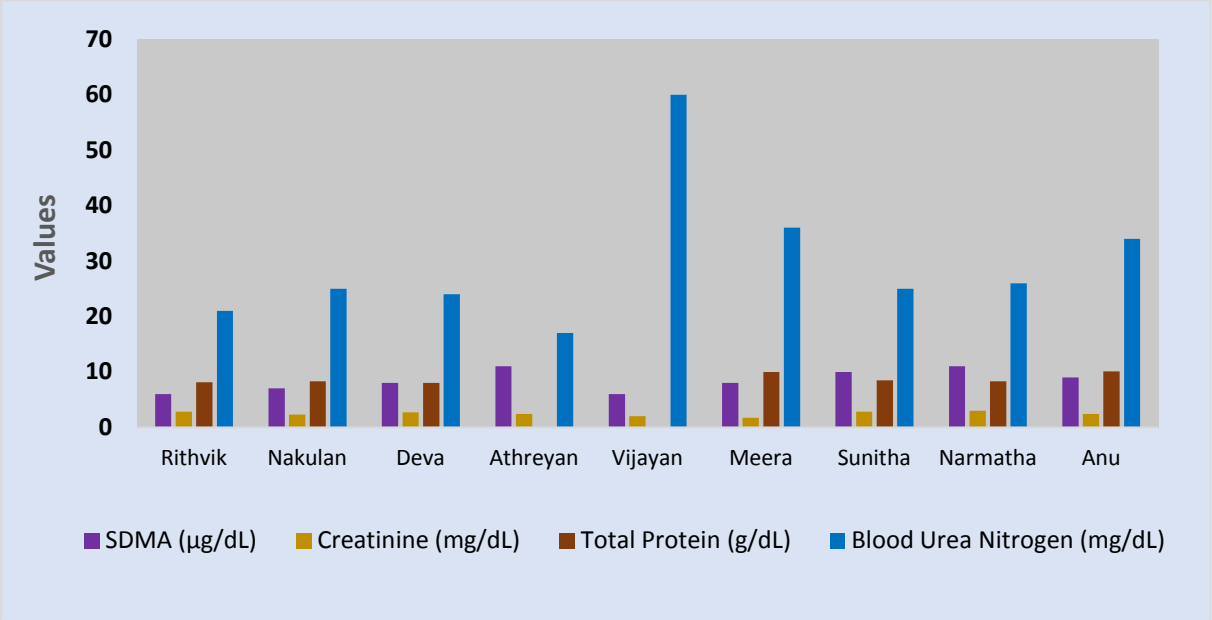

Figure 6. SDMA, Creatinine, Total Protein and Blood Urea Nitrogen in the serum of nine tigers at Arignar Anna Zoological Park

Serum Biochemical Parameters: Table 1 shows the serum biochemical parameters of nine tigers. For the Tiger 'Vijayan' (19 years old/Male), the serum appeared cloudy as shown in Figure 7. This cloudy nature of the serum was due to high levels of cholesterol (371 mg/dL), which was statistically significant ($p<0.05$) compared to all other tigers studied.

Figure 7. Cloudy appearance of serum collected from Tiger 'Vijayan' due to high levels of cholesterol

Blood urea nitrogen was also higher in Vijayan (60 mg/dL) (Fig. 8), which indicated cardiovascular/renal issues due to age factor. As per physical observation, Vijayan had neurological signs, hind leg wobble, and its eyes and ears were not alert but on the whole Vijayan looked fine for his age. Its diet was shifted from red to white meat as a recommendation for the increase in cholesterol.

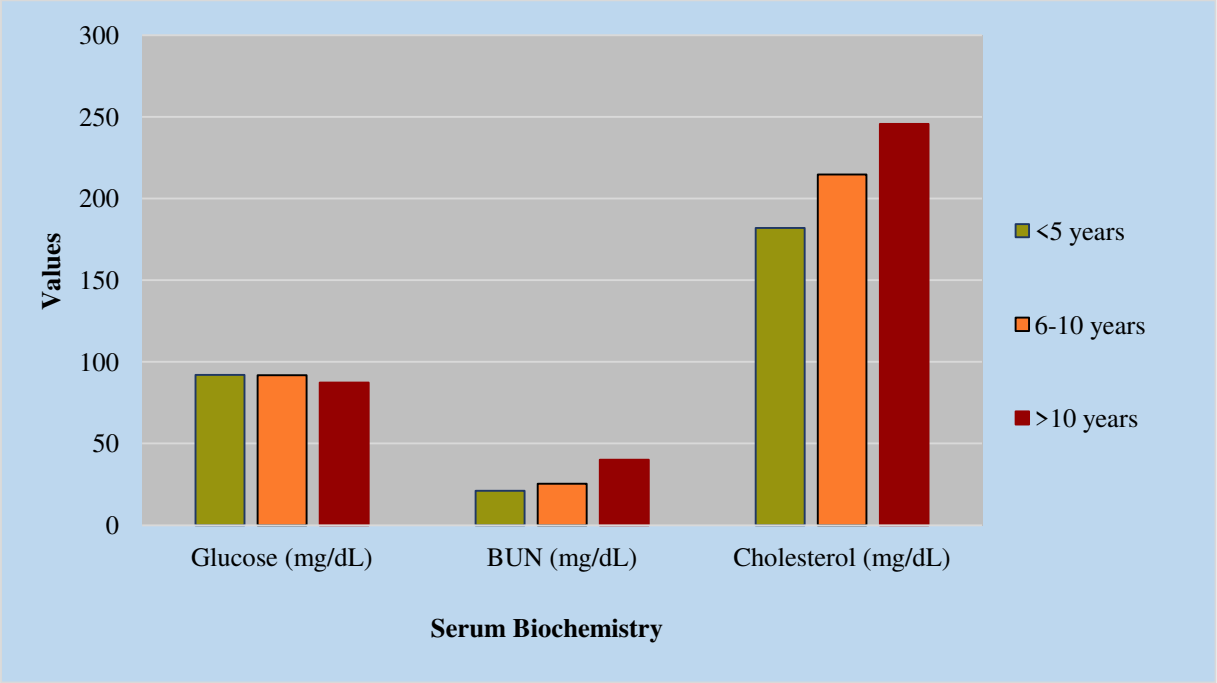

Figure 8. Glucose, Blood Urea Nitrogen and Cholesterol in different age group of tigers

Tiger 'Nakulan' (8 Years/Male) which already had renal issues did not show any significant changes in the serum parameters and falls under the normal reference intervals. Results of Tiger 'Narmatha' (13 Years/Female) also falls under the normal reference interval in spite of its physical examination showing tumour formation, poor wound healing capacity and poor parenting ability. Tiger 'Meera' (8 Years/Female) showed a slight elevation in Total Bilirubin level (1 mg/dL) when compared to other tigers but that was within the normal reference interval. Vijayan showed a peak in blood urea nitrogen while both Vijayan and Athreyan (10 Years/Male) showed low levels of total protein which may indicate a long-term renal dysfunction. Figure 9 shows simple linear models comparing age with SDMA, glucose, creatinine, BUN, cholestrerol and ALT. The linear model figures show that all serum parameters were not affected by the age of the animals.

Table 1. Serum biochemical parameters of Royal Bengal tigers in Arignar Anna Zoological Park (AAZP), Vandalur

S. No.	Parameters	Units	Male					Female			
	Normal/White	**---**	**NT-1**	**NT-2**	**NT-3**	**WT-1**	**NT-4**	**WT-2**	**NT-5**	**WT-3**	**WT-4**
1	**Age**	**Years**	**5**	**7**	**9**	**10**	**18**	**8**	**9**	**13**	**20**
2	**Glucose**	**mg/dL**	92	76	85	109	94	101	88	74	94
3	**SDMA**	**µg/dL**	6	7	8	11	6	8	10	11	9
4	**Creatinine**	**mg/dL**	2.8	2.3	2.7	2.4	2	1.7	2.8	3	2.4
5	**BUN**	**mg/dL**	21	25	24	17	60	36	25	26	34
6	**BUN/Creatinine**		7	11	9	7	30	21	9	9	14
7	**Phosphorus**	**mg/dL**	4.5	5.9	5.3	5.3	7.3	6.7	6.3	6.3	6.2
8	**Calcium**	**mg/dL**	10.1	10.1	10.1	10.4	10.1	10.5	10.0	10	10.4
9	**Total Protein**	**g/dL**	8.1	8.3	8.0	0	0	10	8.5	8.3	10.1
10	**Albumin**	**g/dL**	3.8	3.5	3.7	5.3	3.1	4.4	3.8	3.6	3.6
11	**Globulin**	**g/dL**	4.3	4.8	4.3	0	0	5.5	4.7	4.7	6.6
12	**Alb/Glob**		0.9	0.7	0.9	0	0	0.8	0.8	0.8	0.5
13	**ALT**	**U/L**	51	62	70	16	67	119	95	68	58
14	**ALP**	**U/L**	15	20	21	<10	23	<10	40	21	13
15	**GGT**	**U/L**	0	0	0	2	0	0	0	0	0
16	**Total Bilirubin**	**mg/dL**	0.3	0.1	0.3	0.6	0.3	1	0.3	0.2	0.3
17	**Cholesterol**	**mg/dL**	182	189	202	262	**371**	218	202	155	211
18	**Amylase**	**U/L**	2158	2018	2113	>2500	2216	2378	>2500	2463	2333
19	**Lipase**	**U/L**	129	127	143	38	99	127	130	149	105
20	**Sodium**	**mmol/L**	162	167	161	163	155	164	159	165	160
21	**Potassium**	**mmol/L**	4.4	4.6	4.5	4.9	4.6	5.4	4.2	4.5	5.4
22	**Na/K**		37	37	36	33	34	30	38	36	29
23	**Chloride**	**mmol/L**	119	122	124	121	121	124	120	120	120
24	**Osmolality**	**mmol/kg**	322	333	322	---	323	333	318	329	326

BUN = Blood Urea Nitrogen; Alb/Glob = Albumin/Globulin; ALT = Alanine Aminotransferase; ALP = Alkaline Phosphatase; GGT = Gamma-Glutamyl Transferase; Na/K = Sodium/Potassium

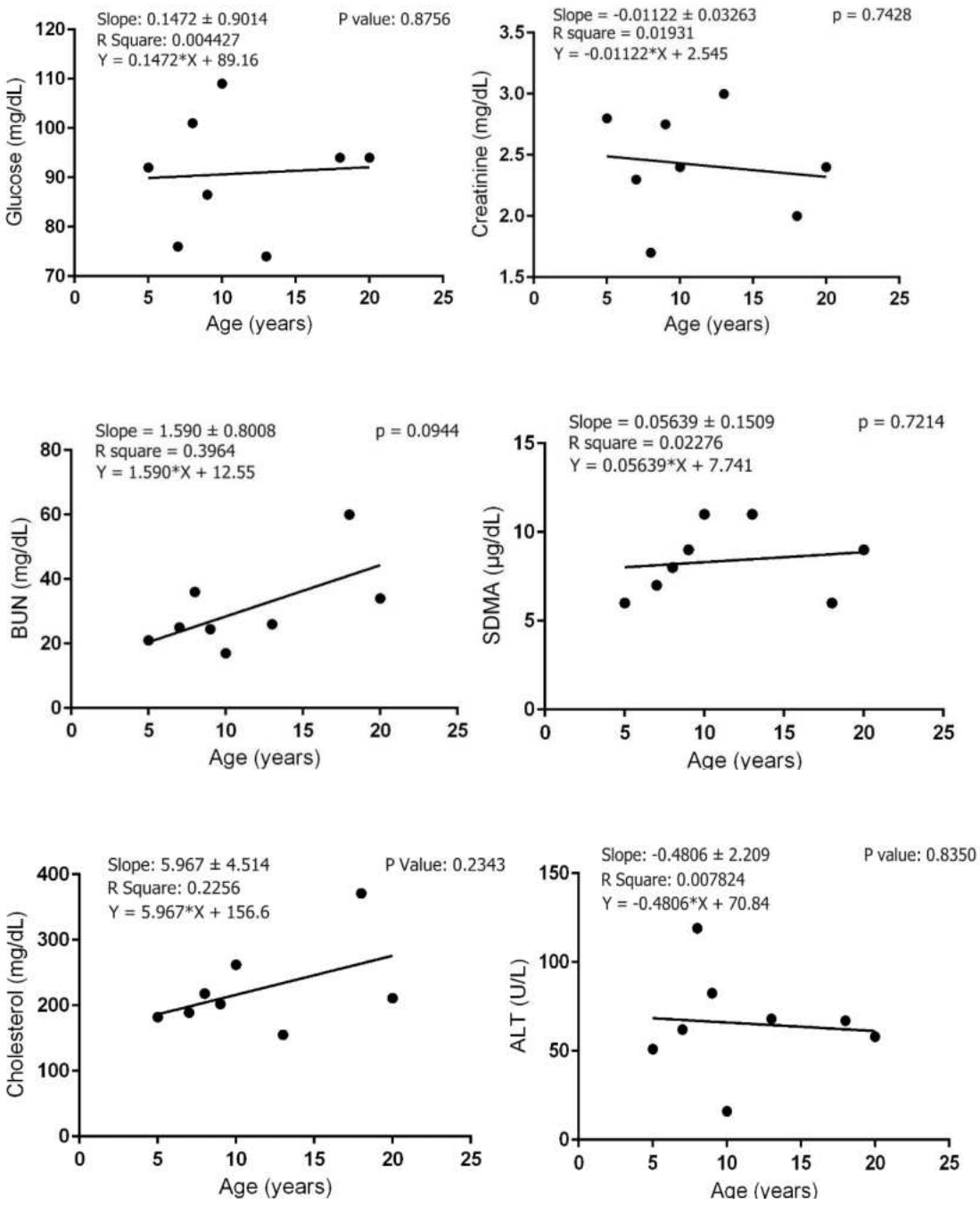

Figure 9. Simple linear model comparing Age and some serum parameters, Glucose, Creatinine, Blood Urea Nitrogen, SDMA, Cholesterol and ALT levels in tigers of AAZP

Haematological Parameters: Table 2 shows the haematological parameters of nine tigers. Total WBC count was slightly higher in males than females. However, the difference was not statistically significant. There was no gender-related difference between RBC and haemoglobin (Fig.10). In general, the haematological parameters were found to be normal in all tigers. Tiger 'Anu' showed the lowest WBC value (5.9 x10^3/µl) while Tiger 'Meera' showed the lowest platelet value (71 x10^3/µl). The rest of the tigers showed normal values in other parameters

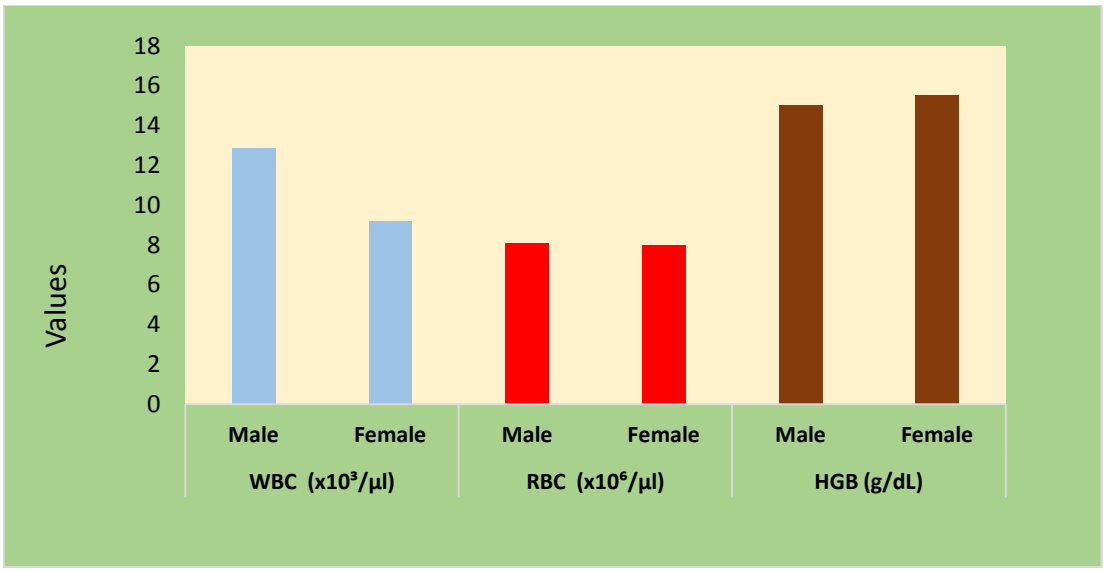

Figure 10. WBC, RBC and Haemoglobin levels in the blood of male and female tigers at Arignar Anna Zoological Park

Table 2. Haematological parameters of Bengal tigers in Arignar Anna Zoological Park (AAZP), Vandalur

Sl. No.	Parameters	Units	Male					Female			
	Normal/White	---	Normal	Normal	Normal	White	Normal	White	Normal	White	White
	Age	Years	5	7	9	10	18	8	9	13	20
1	WBC	($x10^3/\mu l$)	11.2	10.50±2.07	15.4	12.7	14.6	7.1	11.9	11.8	5.9
2	Lymph#	($x10^3/\mu l$)	2.0	2.16±0.23	1.8	1.8	2.5	1.7	2.5	2.0	1.6
3	Mon#	($x10^3/\mu l$)	0.6	0.63±0.11	0.9	0.4	0.8	0.3	0.7	0.6	0.4
4	Gran#	($x10^3/\mu l$)	8.6	7.70±1.73	12.7	10.5	11.3	5.1	8.7	9.2	3.9
5	Lymph%	%	17.7	21.10±2.07	11.8	14.5	17.1	24.1	20.9	17.3	27.6
6	Mon%	%	5.3	6.16±0.28	5.7	3.4	5.8	4.5	5.8	5.3	6.3
7	Gran%	%	77.0	72.73±2.36	82.5	82.1	77.1	71.4	73.3	77.4	66.1
8	RBC	($x10^6/\mu l$)	8.07	7.73±0.66	7.13	8.94	8.47	8.79	7.63	7.09	8.48
9	HGB	g/dL	15.2	14.16±1.84	14.5	16.3	15.1	17	15.5	13.2	16.5
10	HCT	%	44.3	42.13±5.25	42.7	46.9	45.0	48.6	46.0	39.0	48.1
11	MCV	fL	55.0	55.80±0.86	60.0	52.5	53.2	55.4	60.3	55.3	56.8
12	MCH	Pg	18.8	18.23±0.75	20.3	18.2	17.8	19.3	20.3	18.6	19.4
13	MCHC	g/dL	34.3	33.60±0.17	33.9	34.7	33.5	34.9	33.6	33.8	34.3
14	RDW	%	16.4	16.40±0.52	15.6	16.4	19.0	16.1	15.6	16.9	15.0
15	Platelet	($x10^3/\mu l$)	163	109.66±52.53	334	201	149	71	318	274	237
16	MPV	fL	11.3	10.20±0.69	10.5	10.6	10.4	8.3	10.6	11.5	11.1
17	PDW		16.2	16.36±0.46	16.1	16	15.3	17	16.2	15.3	15.7
18	PCT	%	0.184	0.114±0.05	0.350	0.213	0.154	0.058	0.337	0.315	0.263
19	Eos%		1.8	4.60±0.86	1.7	2.0	4.8	3.2	3.6	7.2	12.9

* No. of Replications = 3

WBC = White Blood Cells; **Lymph** = Lymphocyte; **Mon** = Monocyte; **Gran** = Granulocyte; **RBC** = Red Blood Corpuscle; **HGB** = Haemoglobin; **HCT** = Haematocrit; **MCV**= Mean Corpuscular Volume; **MCH** = Mean Corpuscular Haemoglobin; **MCHC** = Mean Corpuscular Haemoglobin Concentration; **RDW** = RBC Distribution Width; **MPV** = Mean Platelet Volume; **PDW** = Platelet Distribution Width; **PCT** = Platelet Crit; **Eos** = Eosinophil

Discussion & Conclusion

Diagnosis of CKD using Creatinine and Blood Urea Nitrogen– the most common biomarkers - have several limitations due to sensitivity and inter-individual variation. SDMA is accepted as a more sensitive and promising biomarker for the early detection of CKD in large felids. SDMA is less affected by extra renal factors. This study aims to estimate the SDMA levels in tigers of AAZP and evaluate the health of the same. In the present study, the analysed SDMA level in nine tigers ranged between 6-11 µg/dL. It was reported that SDMA level below 14 µg/dL can be accepted as a normal range in cats. In all tested tigers in the present study, including some individuals which had health issues at the time of sample collection, the SDMA level was less than 11 µg/dL.

It was reported that SDMA level below 14 µg/dL can be accepted as a normal range in cats (Brans *et al*., 2021) and similar value can also be considered as the optimum SDMA level in large cats like tigers. In Vijayan, the BUN and cholesterol levels were higher but SDMA was below the standard limit. So, all the studied tigers at AAZP were found to be free from chronic kidney diseases. These findings indicate that the captive tigers receive adequate care in both diet and physical activity, as apparent from the normal haematological and serum biochemical readings. Since the blood and serum samples were collected only once, the study should be continued to get more reliable results.

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For Contact

The Principal Chief Conservator of Forests & Director,
Advanced Institute for Wildlife Conservation
(Research, Training & Education),
Tamil Nadu Forest Department, Vandalur,
Chennai – 600 048.

E-mail: aiwcrte@tn.gov.in
Website: www.aiwc.res.in