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Research Article

Biochemical reference values of captive Royal Bengal tigers (*Panthera tigris tigris)* in Orissa, India

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Abstract

The tiger is an important predator in the Asian geographical region. At present only 5000-7000 tigers are left throughout the world and their population is dwindling at a faster pace. It is classified as endangered species and red listed by IUCN. The available biochemical reference values for tigers are limited. So establishing reference values is important for treatment and maintenance of healthy population in wild and captivity. The aim of the study is to establish reference values of serum biochemical parameters of tigers at Nandankanan zoological park, Orissa. Blood samples obtained from lateral coccygeal vein of 18 male and 10 female tigers by the zoo authorities were handed over to Regional centre for wildlife health as part of their program to monitor the health status of the tigers. Clotted blood was centrifuged at zoo veterinary hospital; serum was separated and was analyzed with autoanalyser. Total protein, albumin, urea, creatinine, bilirubin, serum enzymes like ALT, AST, ALP, LDH etc. were analyzed. Results between groups were compared using t-test. Triglycerides, uric acid and creatinine varied significantly between sexes. Total protein, albumin and AST varied significantly between seasons and alkaline phosphatase was significantly higher in younger ones. Associated animal particulars like age, sex, season were taken into consideration to analyse the biochemical reference values with the health status of the tigers.

Keywords: Reference values, serum chemistry, *Panthera tigris*, enzymes, tiger,

Introduction

The tiger is an important carnivore in the Asian geographical region. The tiger has wide distribution and it has variation in appearance to adapt itself to the extreme climatic conditions from cold climate of Siberia to tropical climate of India. At the start of the 20th century, it is estimated there were over 100,000 tigers in the wild, but the population has dwindled outside of captivity to between 1,500 and 3,500. The population of tigers has increased in India from 1,706 in 2011 to 2,226 in 2014, leaving India as home to 70% of the global wild tiger population(1). Although it is one of the most magnificent and revered animal, the tiger is listed as "endangered" on the IUCN Red List of Threatened Species (EN C2a(i)ver 3.1 (2001)) and is also listed on CITES Appendix I, which makes trading of live cats or cat parts (i.e., fur, bones and meat) illegal in signatory countries. Commercial poaching, a decline in prey base due to over hunting,

and loss of habitat are the principal threats to tiger (1, 2). Establishing reference values is therefore important, for their treatment in wild and for maintaining of healthy population in captivity. The available biochemical reference values are limited and those available were either done with a small population size or with few biochemical parameters. However the effect of age, sex and season on biochemical parameters is established in other species (3,4).

The purpose of this study is to establish reference values of clinically important enzymes and biochemical parameters in tigers using readily available methods, and to find out the effect of age, sex and season on these parameters.

Materials and Methods

Blood samples were collected from 18 male and 10 female animals from Nandankanan zoological park, during various seasons of a year. Among the male tigers, 9 were normal colored and 9 were white, and among female tigers, 9 were normal colored and one was white. Both white tigers (Chinchilla) and normal coloured tigers are similiar except the coat colour difference. White tigers are neither separate sub-species nor albino. In tigers, the chinchilla (color inhibitor) gene is recessive to the normal orange color. When two copies of the color inhibitor are inherited, it results in a white tiger (5, 6).

Sample collection and transport

Blood samples were collected from the healthy tigers by the zoo veterinarians in the mornings prior to feeding to prevent diurnal variation and diet induced variations. Animals were selected based on the schedule of the zoo for routine normal health check up. Tigers were restrained using a squeeze cage and around 10ml of blood was collected from lateral coccygeal vein. Blood samples collected were handed over immediately to authorities from Regional centre for wildlife health and Department of Veterinary Biochemistry, Orissa University of Agriculture and Technology. The Regional centre for wildlife health monitored the health status of animals at Nandankanan zoo post sample collection. The zoo record was observed thoroughly to fix the criteria of age, sex, pedigree, physiological and pathological conditions of the animals under study. 1-2ml of blood was transferred to a vial containing sodium fluoride as an anticoagulant for estimation of blood glucose; the rest was transferred to a wide mouth test tube and allowed to clot for 1-2 hours. The clotted blood was centrifuged at zoo veterinary hospital; serum separated was transported in an ice pack to the Regional centre for wildlife health, for further analysis.

Biochemical estimation

Serum enzymes were estimated within four hours of sample collection. Blood glucose analysis was done by Nelson-Somogyi method(7,8), serum calcium by Webster(9) using Perkin-Elmer Spectrophotometer (Perkin-Elmer. Ltd, Becacornsfield, Buchinghamshire, UK) and all other parameters were analyzed with Semiautoanalyser Photometer 5010 (Nicholas piramal Pvt. Ltd., Worli Naka, Lower Parel, Mumbai) using Ecoline® kits (Merck Limited, M.I.D.C Area, Ambernath-421501).

Data analysis

The obtained data were tested for normality using mean ± 5 (SD) as a standard, and those which fell below the level were taken as outliers. None of the values fell beyond this level in our study. Comparison between male and female were made using student t-tests, to find out any significant variation at $P<0.05$ and $P<0.01$ levels using Microsoft Excel 2003 software (Microsoft, Redmond,Wa, USA).

Results

Serum chemistry values were reported in table-1 and 2. Triglycerides showed significantly higher values at $P<0.01$ levels. The mean serum triglyceride value obtained is 68.45±3.77 mg/dl, with male being significantly higher than female. Aspartate transaminase showed a significant difference with the female at higher levels at $P<0.05$ level. Total protein, albumin, globulin, urea, creatinine, cholesterol, ALT, AST and LDH were higher in females though not significant. Blood urea, cholesterol and LDH had wide ranges, 78.08-164.49 IU/L, 84.69-240.2 IU/L and 98.65-267.1 IU/L respectively. Total protein, albumin and aspartate transaminase had significant differences between seasons and alkaline phosphatise was significantly higher in below 5 years age group.

Table-1. Royal Bengal Tigers biochemical reference values

PARAMETER	*Overall(n=28*	*Male(n=18)*	*Female(n=10*	*Range*	*P*
GLUCOSE (mg/dl)	84.48±1.54	85.83±1.91	82.06±2.57	71.48-97.54	0.250
TOTAL PROTEIN	7.25±0.12	7.18±0.14	7.37±0.21	5.38-8.3	0.458
ALBUMIN (g/dl)	3.89±0.10	3.83±0.13	4.01±0.15	2.52-4.77	0.407
GLOBULIN (g/dl)	3.35±0.09	3.35±0.11	3.36±0.16	2.01-4.17	0.962
BLOOD UREA (mg/dl)	123.22±4.45	119.52±6.19	129.87±5.31	78.08-164.49	0.273
CREATININE (mg/dl)	1.58±0.04	1.57±0.05	1.62±0.07	1.33-2.24	0.621
URIC ACID (mg/dl)	0.90±0.08	1.07±0.09*	0.60±0.10*	0.32-1.8	0.003
BILIRUBIN (mg/dl)	0.75±0.10	0.80±0.13	0.65±0.15	0.09-1.9	0.492
CHOLESTEROL(mg/d	159.85±7.27	157.43±9.85	164.20±10.55	84.69-240.2	0.664
TRIGLYCERIDE(mg/	68.45±3.77	74.72±5.22*	57.18±2.17*	45.1-117.63	0.022
CALCIUM(mg/dl)	11.5±0.39	11.7±0.48	11.0±0.67	7.53-14.7	0.357
ALT (IU/L)	27.52±2.18	26.01±2.87	30.24±3.29	9.29-48.69	0.364
AST (IU/L)	19.76±1.55	16.91±1.60*	24.88±2.65*	6.53-35.7	0.011
ALP (IU/L)	19.86±1.68	19.96±2.27	19.70±2.50	5.22-34.36	0.942
LDH (IU/L)	147.44±8.01	138.76±9.38	163.08±14.07	98.65-267.1	0.149

NOTE: *Superscript bearing single asterisk is significant at (P<0.05) level between the groups. n: Number of animals. P- P indicates P value*

Table-2 Royal Bengal Tigers biochemical reference values between seasons and age

PARAMETER	*Summer*	*Winter*	*<5 Year age*	*>5 Year age*
GLUCOSE (mg/dl)	86.09±2.04	83.09±2.28	85.96±2.56	83.89±1.92
TOTAL PROTEIN (g/dl)	7.56±0.11*	6.98±0.17*	7.17±0.13	7.28±0.16
ALBUMIN (g/dl)	4.12±0.12*	3.69±0.14*	3.65±0.14	3.99±0.12
GLOBULIN (g/dl)	3.43±0.09	3.28±0.15	3.52±0.05	3.28±0.12
BLOOD UREA (mg/dl)	123.23±6.05	123.20±6.64	128.58±5.74	121.07±5.80
CREATININE (mg/dl)	1.58±0.06	1.59±0.06	1.51±0.05	1.61±0.05
URIC ACID (mg/dl)	0.83±0.10	0.97±0.12	0.87±0.12	0.92±0.10
BILIRUBIN (mg/dl)	0.90±0.15	0.62±0.13	0.66±0.16	0.79±0.13
CHOLESTEROL(mg/dl)	163.00±9.66	157.12±10.97	177.46±14.99	152.81±7.94
TRIGLYCERIDE(mg/dl)	61.97±3.39	74.08±6.15	65.45±3.44	69.66±5.12
CALCIUM (mg/dl)	12.18±0.44	10.95±0.59	12.24±0.51	11.23±0.50
ALT (IU/L)	27.69±3.21	27.37±3.09	28.59±3.23	27.10±2.82
AST (IU/L)	23.51±2.38*	16.51±1.68*	22.60±2.84	18.62±1.83
ALP (IU/L)	20.18±2.03	19.59±2.67	26.57±1.59*	17.18±1.99*
LDH (IU/L)	133.45±8.58	159.58±12.41	136.27±8.94	151.91±10.60

NOTE: *Superscript bearing single asterisk is significant at (P<0.05) level between the groups. n: Number of animals. P- P indicates P value*

Discussion

The values of total serum proteins were within the ranges/ mean values reported by previous workers in different tiger sub species (10, 11, 12) and the average values also matches with that of lions (13) and jaguars (14). The differences in total serum protein and albumin values between juveniles and adults could also be explained as an age related phenomenon, having been observed in Florida panther (15).

Our values obtained for blood urea was higher than the findings of other workers (10, 11) in tiger. As per their report the blood urea measures between 32.76 to 115.44 mg/dl. In the present study, we observed higher values of urea than the reported value in the tigers below 5 years of age. These results were similar to that observed in Canadian lynx(16). But the findings in bobcats (17) and mountain lions (18) contradict our results. Blood urea values are also influenced by protein content in diet (19). If the diet contains more amount of protein, the level of urea increases in blood, but in the present study the influence of protein for higher urea level in diet content may not be of significance because whether it is wild or captive, tigers depend only on meat. Therefore the increase may be attributed to regular supply of meat to the captive tigers compared to the wild ones.

There is significant difference between male and female tigers, in case of uric acid, with the males having higher values. Our findings were in parallel with the findings in bobcats where males had higher values(20). The total cholesterol is non-significantly higher in case of females, which is similar to what is observed in irimote cats of Japan(21) where the females had higher cholesterol values. The observed range complied with the range for tigers as reported by other workers(10,11).

The activity of alkaline phosphatase ranged from 5.22 to 34.36IU, with a significant decrease in tigers above 5 years of age. The observed values are within the range specified by ISIS reference values(10). This is the phenomenon observed between middle-aged and older bears(22). Alkaline phosphatase exists in different isoenzymes and is responsible for the hydrolysis of monophosphate esters in different tissues. The isoenzyme from bone is produced by osteoblasts and blood levels may be three times higher in young and rapidly growing animals compared to adults (23) which corresponds with our findings and those of free-ranging polar bears in Canada (24), and in other species like bullock, cattle. This is similar to what was observed in ISIS data (10) for tiger. Serum ALP activity is known to be concerned with mineral deposition in bone. ALP activity might be higher in younger age group due to active bone formation during growth than adult.

Aspartate transaminase values showed significant difference between summer and winter. Higher values were observed in summer. This is in contrary with the observations in foxes in which higher values were observed in winter and they attributed the reason to extrahepatic tissue damage (25). Also in, Canadian lynx (16), mountain lions(18), bob cats(20) and Iberian lynx(26) there is increase in AST values due to stress during capture. During summer the temperature in Orissa is very high. So the increase in values of Aspartate transaminase observed in summer in our study may be associated with the summer stress and its consequent tissue damage in the tigers of this region.

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References

1.Y. V. Jhala, Q. Qureshi, and R. Gopal (eds) 2015. The status of tigers in India.. National Tiger Conservation Authority, New Delhi & The Wildlife Institute of India, Dehradun. 2014.
2. Seidensticker J, Christie S, Jackson P. Riding the tiger: Tiger conservation in human-dominated landscapes. Cambridge University Press, Cambridge, UK 1999.

3. Cornish, HH. Problems posed by observations of serum enzyme changes in toxicology. CRC Critical Reviews in Toxicology. 1971;1: 1-32.
4. Bush M, Smith EE, Custer RS. Hematology and serum chemistry values for captive dorcas gazelles: variations with sex, age and health status. Journal of Wildlife Diseases 1981; 17(1):135-143.
5. Robinson, Roy. Robinson's Genetics for Cat Breeders and Veterinarians. Butterworth Heinemann. 4th Ed. 1999.
6. A C Jude Cat Genetics. Tfh Pubns Inc 1977.
7. Nelson P, Yarnell G, Walle SR. Archs biochem. Biophys. 1966;114:543-546.
8. Somogyi M. J. Biol. chem. 1945;160:61-68.
9. Webster Jr WW. American J. Clin. Path. 1962;37: 330-333.
10. ISIS physiological Data Reference values. Normal blood values for Panthera tigris (1995).Available at www.5tigers.org accessed. September 2004.
11. Fowler M.E. Zoo and Wild Animal Medicine. 2nd Edn. W. B. Saunders Co. Philadelphia. 1986
12. Tilson RL, Seal VS. The tigers of the world, Noyes Publications. New Jersey, U.S.A 1987.
13. Christi KS, Sabapara RH, Vadodaria VP. Certain haematological and bio-chemical profiles in Asiatic lioness (Panthera leo persica). Zoo's Print. 1998;13(3):17-19.
14. Deem SL. Capture and immobilization of free-living Jaguars (Panthera onca). International Veterinary Information Service, Ithaca, NY 2002
15. Lowseter LA,Gillet NA, Gerlach RF, et al. The effects of aging on hematology and serum chemistry values in the beagle dog. Veterinary Clinical Pathology 1990;19:13-19.
16. Weaver JL, Johnson R. Hematologic and serum chemistry values of captive Canadian lynx. Journal of Wildlife Diseases 1995;31: 212–215.
17. Fuller TK, Kerr KD, Karns PD. Hematologic and serum chemistry of bobcats in north central Minnesota. Journal of Wildlife Diseases. 1985;21: 29–32.
18. Currier MJP, Russell KR. Hematology and blood chemistry of the mountain lion (Felis concolor). Journal of Wildlife Diseases 1982;18: 99–104
19. Marco I, Martinez F, Pastor J, et al. Hematologic and serum chemistry values of the captive european wildcat. Journal of Wildlife Diseases, 2000; 36(3):445–449.
20. Kocan AA, Blouin EF, Bertis L. Hematologic and Serum Chemical Values for Free-ranging Bobcats, Felis rufus (Schreber), with Reference to Animals with Natural Infections of Cytauxzoon felis Kier, 1979. Journal of Wildlife Diseases, 1985;21(2):190-192.
21. Fushuku S, Yasuda N, Matsumoto M, Izawa M, et al. Reference Values and Limited Serological Survey for the Iriomote Cat in Japan. Journal of Wildlife Diseases. 2001;37(3):653–656.
22. Tryland M, Brun E, Derocher AE, Arnemo JM, et al. Plasma biochemical values from apparently healthy free-ranging polar bears from Svalbard. Journal of Wildlife Diseases, 2002;38(3):566–575
23. Duncan JR, Prasse KW. Veterinary laboratory medicine, clinical pathology. The Iowa State University Press, Ames, Iowa, 1983;243
24. Lee J, Ronald K, Oritsland NA. Some blood values of wild polar bears. Journal of Wildlife Management. 1977;41:520–526.
25. Patrick M, McCue'2, Farrell TPO. Serum chemistry values of the endangered San Joaquin kit fox (vulpes macrotis mutica). Journal of Wildlife Diseases, 1992;28(3): 414-418
26. Beltran JF, Delibes M, Recio F, et al. Hematological and serum chemical characteristics of the Iberian lynx (Lynx pardina) in southwestern Spain Canadian Journal of Zoology 1991;69: 840–846.