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Haematological profiles of royal Bengal tigers in India: Effect of age and sex

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Abstract
The present study was conducted to establish the age and sex wise haematological profile of Royal Bengal tigers in India. The tigers maintained in Bannerghatta Biological Park (BBP), Bengaluru, Karnataka, India were employed for the present study. Tigers were housed and fed as per the standard feeding practice. The blood samples collected from 33 tigers (16 males and 17 females) were subjected to haematological estimation. Males and females were separately grouped as Group - 1 (ie. less than 4 years of age or before puberty), Group - 2 (ie., between 4 - 6 years of age or at puberty) and Group - 3 (ie., more than six years of age or after puberty). The differential leukocyte count (DLC) of all giemsa stained slide samples was done manually. The results of haematological parameters when compared between age groups revealed significant difference only for hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and lymphocytes. And also mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration shows significant difference when compared between sexes. These data provides baseline information of haematological variation in Royal Bengal tigers with respect to different age (pre pubertal, pubertal and post pubertal) and with respect to sex which we be helpful for therapeutic evaluateon and monitoring of Royal Bengal tigers kept in captivity.

Keywords: Haematological, age wise, sex wise, Royal Bengal tigers

Introduction
Tiger (*Panthera tigris*) is the largest big cat and classified as endangered by International Union for Conservation of Nature (Jhala *et al*., 2015; Krishnan *et al*., 2015; Sajjad *et al*., 2012) [4, 5, 7]. The cause for extinction is postulated to be poaching, decline in pery base, nutritional deficiencies and/or infectious diseases. Hence, proper health monitoring / management, disease diagnosis and treatment becomes essential for conservation of tiger population (Shrivastav and Singh, 2012) [9]. In addition, hematological norms of the tissue is essential for understanding a pathological entity has been put forth (Simsek *et al*., 2015) [10] and comprehensive data of the same in Bengal tigers is yet to be established. In the view of absence of comprehensive study with respect to age and sex on haematological of Bengal tigers, the present work is being envisaged in Royal Bengal tigers. Haematological profiles reflecting health status of animal includes red blood cells which reflecting oxygen carrying capacity is said to revolve around its Hb content as well as its indices *viz*., mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), its constituent and its indices, leukocytes includes neutrophils, lymphocytes, eosinophils, monocytes and basophils that have role in first line defence, infection, allergy, phagocytosis and synthesis of heparin respectively and its different types as well as platelets.

Materials and Methods
The tigers maintained in Bannerghatta biological park (BBP), Bangaluru, were employed for the present study. All tigers were housed and fed as per the standard feeding practice. They were housed in separate cages in zoo (BBP safari) with free access to an outside area during the day time.
The total of 33 tigers was used for the present study. They were grouped with respect to their age as Group - 1 (ie. less than 4 years of age or before puberty), Group - 2 (ie., between 4 - 6 years of age or at puberty) and Group - 3 (ie., more than six years of age or after puberty). The number of males was 16. They were grouped with respect to their age as Group - 1, Group - 2 and Group - 3 and the number of tigers in each of these groups were three, six and seven respectively. Likewise, the number of females was 17.

They were also grouped with respect to their age as Group - 1, Group - 2 and Group - 3 and the number of tigers in each of these groups were three, four and ten respectively.

Collection and Processing of Blood Samples

The blood samples in triplicates from all the tigers (16 males and 17 females) were obtained from the samples (for regular screening / treatment) being collected by the veterinary officer in charge of zoo from tail vein after restraining the animal in squeeze cage. The blood samples obtained being immediately taken in vial containing ethylene diamine tetre acetic acid (EDTA) for haematological analysis [total erythrocyte count (TEC), packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (TLC), total platelet and mean platelet volume (MPV)] by employing automated hematology analyser (Model: Huma Count-30TS, including veterinary software). The differential leukocyte count (DLC) of all giemsa stained slide samples was done manually after giemsa staining as described by Brown *et al*. (1993) [1]. A drop of each blood sample was taken on individual slide, thin smear was made and fixed with methanol for five minutes, air dried. Subsequently diluted giemsa stain (1:20 with deionized water) was used to stain the blood smear. The smear was allowed to stand for 30 minutes and washed with distill water, air dried and visualised under 100x oil immersion.

Statistical analysis

The results obtained for all three age groups (ie., Group - 1, Group - 2, and Group - 3) in both male and female tigers were subjected for statistical analysis by two way ANOVA using computerized prism software (Graph Pad prism 6). The statistical analysis was done to compare the mean value of each of the biochemical and haematological parameters assayed between Group - 1, Group - 2 and Group - 3 in both male and females. Comparison was also done between sex within each of the three age groups. Further the statistical analysis wherever found non-significant when analysed between age groups or between sex was analysed for difference in pooled values irrespective of age in both male and female and also for pooled values irrespective of sex for all the three age groups. The significance is estimated at $P \leq 0.05$.

Results and Discussion

The mean of haematological data in male and females tigers of of Group - 1, Group - 2 and Group - 3 as well as that for pooled samples irrespective of age for male and female tigers and pooled samples irrespective of sex for Group - 1, Group - 2 and Group - 3 tigers are shown in Table 1 and 2.

The highest and lowest mean hemoglobin value in male and female tigers of Group - 1, Group - 2 and Group - 3 was 15.77 ± 1.03 and 12.92 ± 1.15 g/dl. The highest and lowest mean MCH value in male and female tigers of Group - 1, Group - 2 and Group - 3 was 19.50 ± 0.46 and 17.21 ± 0.23 pg. The highest and lowest mean MCHC value in male and female tigers of Group - 1, Group - 2 and Group - 3 was 34.23 ± 0.72 and 29.07 ± 0.84 per cent. The highest and lowest mean lymphocytes value in male and female tigers of Group - 1, Group - 2 and Group - 3 was 29.25 ± 4.21 and 12.67 ± 2.40 per cent. The results of haematological parameters when compared between age groups revealed significant difference only in hemoglobin, MCH, MCHC and lymphocytes, while the value of only MCH and MCHC was found to be significantly different when compared between sex.

Table 1: Haematological constituents in Bengal tigers (Mean ± SE)

Parameter	Age Sex	< 4 year old (Group - 1)	4 - 6 year old (Group - 2)	> 6 year old (Group - 3)	Pooled
TEC (x10 ^{6}/µl)	Male	8.04 ± 0.35	7.74 ± 0.17	8.07 ± 0.43	7.94 ± 0.20
	Female	8.80 ± 0.87	7.89 ± 0.98	7.66 ± 0.20	7.92 ± 0.29
	Pooled	8.42 ± 0.46	7.80 ± 0.37	7.83 ± 0.21	7.93 ± 0.16
PCV (%)	Male	45.90 ± 2.19	43.65 ± 0.76	44.93 ± 2.33	44.63 ± 1.09
	Female	50.80 ± 4.97	44.00 ± 4.03	43.84 ± 1.31	45.10 ± 1.50
	Pooled	48.35 ± 2.67	43.79 ± 1.53	44.29 ± 1.19	44.87 ± 0.93
Hemoglobin (g/dl)	Male	15.77 ± 1.03	13.88 ± 0.28	13.41 ± 0.43	14.03 ± 0.35
	Female	15.03 ± 1.80	12.92 ± 1.15	14.05 ± 0.64	13.96 ± 0.54
	Pooled	15.40 ± 0.94^{a}	13.50 ± 0.47^{b}	13.79 ± 0.42^{ab}	13.99 ± 0.32
MCV (FL)	Male	57.10 ± 0.38	56.48 ± 0.56	55.83 ± 0.84	56.31 ± 0.43
	Female	57.30 ± 0.89	57.42 ± 1.32	57.30 ± 0.72	57.32 ± 0.52
	Pooled	57.20 ± 0.43	56.86 ± 0.60	56.69 ± 0.56	56.83 ± 0.34
MCH (PG)	Male	19.50 ± 0.46^{a1}	17.92 ± 0.22^{b1}	17..21 ± 0.23^{b1}	17.90 ± 0.26
	Female	18.20 ± 0.91^{a1}	17.42 ± 0.28^{a1}	18.31 ± 0.36^{a2}	18.08 ± 0.27
	Pooled	18.85 ± 0.54	17.72 ± 0.18	17.86 ± 0.26	17.99 ± 0.19
MCHC (%)	Male	34.23 ± 0.72^{a1}	31.77 ± 0.30^{b1}	30.93 ± 0.42^{b1}	31.86 ± 0.38
	Female	29.07 ± 0.84^{a2}	31.00 ± 0.71^{ab1}	32.32 ± 0.58^{b1}	31.43 ± 0.49
	Pooled	31.65 ± 1.25	31.46 ± 0.34	31.75 ± 0.41	31.46 ± 0.31
TLC (10^3/µl)	Male	16.37 ± 0.78	16.37 ± 0.93	14.93 ± 0.72	15.73 ± 0.50
	Female	15.82 ± 3.10	14.78 ± 1.51	16.09 ± 1.67	15.73 ± 1.12
	Pooled	16.09 ± 1.43	15.73 ± 0.81	15.61 ± 1.01	15.73 ± 0.62
Neutophils (%)	Male	81.67 ± 4.91	71.67 ± 3.53	81.14 ± 3.21	77.68 ± 2.33
	Female	75.00 ± 8.08	67.00 ± 4.36	76.20 ± 1.84	73.82 ± 2.08
	Pooled	78.33 ± 4.48	69.80 ± 2.70	78.23 ± 1.75	75.69 ± 1.57
Lymphocyte (%)	Male	12.67 ± 2.40^{a}	25.33 ± 3.67^{b}	14.42 ± 2.68^{a}	18.18 ± 2.26
	Female	21.00 ± 6.66^{a}	29.25 ± 4.21^{a}	20.40 ± 1.93^{a}	22.58 ± 1.96
	Pooled	16.83 ± 3.67	26.90 ± 2.67	17.94 ± 1.70	20.45 ± 1.52
Eosinophil (%)	Male	3.67 ± 2.67	2.10 ± 0.36	4.14 ± 1.27	3.25 ± 0.73
	Female	3.33 ± 0.88	3.25 ± 1.03	2.70 ± 0.59	2.94 ± 0.43
	Pooled	3.50 ± 1.26	2.53 ± 0.48	3.29 ± 0.63	3.09 ± 0.41

Monocyte (%)	Male	1.67 ± 0.33	0.33 ± 0.21	0	0.56 ± 0.18
	Female	1.00 ± 0.58	0.50 ± 0.50	0.70 ± 0.26	0.71 ± 0.21
	Pooled	1.33 ± 0.33	0.40 ± 0.22	0.53 ± 0.17	0.64 ± 0.14
Basophil (%)	Male	0.33 ± 0.33	0.67 ± 0.33	0	0.31 ± 0.15
	Female	0	0	0	0
	Pooled	0.17 ± 0.17	0.40 ± 0.22	0	0.15 ± 0.08
Platelet 10^3/µl	Male	208.67 ± 33.32	240.83 ± 9.82	251.53 ± 12.32	239.50 ± 9.07
	Female	329.33 ± 78.38	294.75 ± 34.99	262.90 ± 21.02	283.18 ± 19.41
	Pooled	269.00 ± 46.65	262.40 ± 16.53	258.24 ± 13.10	261.45 ± 11.40
MPV (FL)	Male	9.93 ± 0.41	10.32 ± 0.16	10.17 ± 0.25	10.18 ± 0.14
	Female	9.40 ± 0.12	9.49 ± 0.43	9.90 ± 0.18	9.72 ± 0.15
	Pooled	9.67 ± 0.22	9.99 ± 0.23	10.01 ± 0.15	9.94 ± 0.11

* Superscript bearing different alphabet within the row indicates significant difference between the means.
* Superscript bearing different numericals within the coloumn indicates significant difference between the means.

Hemoglobin

The hemoglobin values so obtained are within a range that have been put forth by Seal *et al*. (1987) [8] and Chandranaik *et al*. (2006) [2] in Bengal tiger. Further, there exists no comprehensive report on changes in hemoglobin with respect to age (i.e., young age, at pubertal age and in adult) within sex as well as with respect to sex (i.e., in male and female) within an age group. In the present study the hemoglobin concentration in males and females of all three age group revealed for no significant difference. Further. There existed no significant difference in the hemoglobin content when observed between male and female within an age groups. However, in the sex-wise pooled data, Group - 2 animals shown significant lower value compared to Group - 1 animals. In contrary to the observations of Thomsen *et al*. (1986) [12], who observed in human males an increase in testosterone causes increased formation of hemoglobin and in females at puberty there will be no differentiation from infants or the adults. In the present study, the reason behind the significant decrease in the pooled data of hemoglobin concentration in Group - 2 (animals at puberty), is not known.

MCH

The MCH values so obtained are within a range that have been put forth by Seal *et al*. (1987) [8] and Larsson *et al*. (2015) [6] in Bengal tiger and Siberian tigers respectively. Further, there exists no comprehensive report on changes in MCH with respect to age (i.e., young age, at pubertal age and in adult) within sex as well as with respect to sex (i.e., in male and female) within an age group. In the present study, MCH follows the pattern of hemoglobin as both the parameters are interrelated wherein the MCH value in male tigers of the Group - 2 was found to be significantly lower and with in Group - 3 the values are significantly higher in females than that of males, for the reasons unknown.

MCHC

The MCHC values so obtained are within a range that have been put forth by Sajjad *et al*. (2012) [7] in Bengal tigers, as well as Larsson *et al*. (2015) [6] and Singh *et al*. (1999) [11] tigers. Further, there exists no comprehensive report on changes in MCHC with respect to age (i.e., young age, at pubertal age and in adult) within sex as well as with respect to sex (i.e., in male and female) within an age group. In the present study, MCHC follows the pattern of hemoglobin and MCH as the three parameters are interrelated wherein the MCH values in male tigers of the Group - 2 was found to be significantly lower than Group - 1; in females the MCHC values are significantly higher in Group - 3 and within Group - 1 the values are significantly higher in males than that of females, for the reasons unknown. Though there lies significant differences in the values of hemoglobin, MCH and MCHC among males, among females, among different groups and with in pooled data, all the values are with in normal physiological reference values given by other authors in Royal Bengal Tigers.

Lymphocytes

The lymphocyte values so obtained are similar to the report that have been put forth by Seal *et al*. (1987) [8] in Bengal tiger and Larsson *et al*. (2015) [6] in Siberian tigers as well as lower than the reports of Chandranaik *et al*. (2006) [2] as well as Shrivastav and Singh, (2012) [9]. Further, there exists no comprehensive report on changes in lymphocyte with respect to age (i.e., young age, at pubertal age and in adult) within sex as well as with respect to sex (i.e., in male and female) within an age group. In the present study, the observation of significantly higher level of lymphocytes in Group - 2 male as well as nonsignificant increase of the same in Group - 2 male tigers indicates that tigers at the age of puberty are getting immunologically acclimatize at puberty.

Conclusion

The obtained data revealed that the concentration of hemoglobin, MCH, MCHC and lymphocyte concentration, which are influenced by the growth, physiological variation and body response to the environment and the rest of the haematological parameters are not influenced by age and sex in Royal Bengal Tigers.

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