

Haemogram and Serum Biochemical Values of Four Indigenous Species of Monkeys in South West Nigeria

Ogunro B. N.^{1*}, Otuh P. I.¹, Ogunsola J. O.¹, Kolawole O. N.², Jegede H. O.³,
Makinde O.⁴, Ademakinwa J.⁵, Morenikeji O.⁶

¹Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Ibadan, Oyo state, Nigeria. ²Zygos (Nig.) Limited, Benin city, Edo state, Nigeria. ³Veterinary Teaching Hospital, University of Ilorin, Kwara state, Nigeria. ⁴Osun Osogbo Sacred groove, Osogbo, Osun state, Nigeria. ⁵Veterinary Clinical Pathology Laboratory, Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Oyo state, Nigeria. ⁶Department of Zoology, Faculty of Science, University of Ibadan, Oyo state, Nigeria

Summary: Haematological and serum biochemical values are useful guides and biomarkers in health and diseases for reaching a diagnosis, estimating disease prognosis and monitoring treatment progress, in mammals. Reference ranges for some parameters differ among species of mammals and between sexes within a species. There is dearth of information on standard reference value for blood parameters for Nigerian indigenous monkeys. Whole blood and serum samples obtained from 50 apparently healthy adult monkeys in both captivity and from the wild in southwest Nigeria were subjected to haematology and serum biochemistry to obtain preliminary reference values for haematological and serum biochemical analytes for *Cercocebus sebaeus* (Green monkey), *Cercopithecus mona* (Mona monkey), *Erythrocebus patas* (Patas monkey) and *Papio anubis* (Anubis baboon). Numerical data were summarized as mean and standard deviation and subjected to statistical analysis; Student t test and analysis of variance, to compare values of blood parameters obtained between species and gender. A p-value less than 0.05 was considered significant. The hematocrit of male animals were significantly higher than that of females (P=0.01) in all the 4 species studied but there was no significant difference in other blood parameters such as total white blood cell and the differential counts, platelet count, serum aspartate transferase, alanine transferase, alkaline phosphatase, total plasma protein, albumin, and globulin concentrations between the sexes. Generally, there was no significant difference between total white blood cell and the differential counts, hematocrit, red cell count, haemoglobin concentration, platelet count, serum aspartate transferase, alanine transferase, alkaline phosphatase, total plasma protein, albumin, and globulin concentrations among the monkey species.

Keywords: Haematology, Monkeys, Reference data, Serum biochemistry.

©Physiological Society of Nigeria

*Address for correspondence: ogunrob@gmail.com

Manuscript Accepted: February, 2019

INTRODUCTION

Haematological and serum biochemical parameters are useful indicators of health and ill-health in humans and animals (Milner et al., 2003; Bhatia et al., 2004). They are useful guides for reaching a diagnosis, estimating disease prognosis and monitoring treatment progress (Bhatia et al., 2004; Abro et al., 2009). When non-human primates (NHPs) are used in biomedical research, normal reference values for haematological and serum biochemical parameters are often needed for comparison with experimental outcomes (McPherson, 2013). Reference value range for blood parameters often differ between species of animals and significant differences may be noticed between sexes and age groups within a species (Xie et al., 2013; Castro et al., 2016). Diet and environmental influences

also affect the normal blood parameter values in NHPs (McPherson, 2013). The green monkey has been used extensively for biomedical research and therefore has received enormous attention, including basic reference value, unlike other breeds of indigenous monkeys in Nigeria with limited data (reference value to biomarker of health) but also receive a wide use in research (Kagira et al., 2007; Chichester et al., 2015; Castrol et al., 2016). There are currently no standard reference values for blood parameters in Nigerian indigenous monkeys (Mona monkey, Patas monkey, Anubis baboon, etc). Hence, as part of a region wide serosurveillance for antibodies to zoonotic diseases among NHPs (August 2015 to February 2017), whole blood and serum samples obtained from 50 apparently healthy adult (>5years old) monkeys in southwest

Nigeria were subjected to haematology and serum biochemistry to present preliminary reference data for haematological and serum biochemical analytes for 4 indigenous species of monkeys.

MATERIALS AND METHODS

Study sites and animal sampling

A total of 50 whole blood and serum samples were obtained from adult (>5years) individuals belonging to 4 species of captive and wild NHPs in 5 locations within southwest Nigeria: Zoological garden, University of Ibadan, Oyo State (7.4434°N, 3.8956°E); Biological garden, University of Ilorin, Kwara State (8.4817°N, 4.6382°E); Agodi gardens, Ibadan, Oyo State (7.4069°N, 3.8994°E); Osun Osogbo sacred groove, Osun State (7.7592°N, 4.5569°E); and University of Lagos, Akoka campus (6.5155°N, 3.3878°E). Domestic (pet) monkeys in Ibadan, Oyo State, whose owners consented to the sampling of their animals, were also enrolled in the study. Table 1 shows the distribution of sampled animals among the various study sites. The four Mona monkeys (*Cercopithecus mona*) from Osun Osogbo sacred groove and one out of the four Mona monkeys from University of Lagos Akoka campus were trapped directly from the wild. Other types of monkeys sampled were Green monkeys (*Cercocebus sebaeus*), Patas monkeys (*Erythrocebus patas*), and Anubis baboons (*Papio anubis*). Age estimation of the animals was based on curator records in the host facility and date of acquisition of pet animals. Attempts were made at sampling as many NHPs as permitted by curators at the various locations. All animals were apparently healthy at time of sampling and they showed no signs of illness up to three months post sampling. Caged monkeys were darted at close range (1-5m) with a blow pipe delivering anaesthetic (ketamine hydrochloride) at 10mg/kg body weight of the monkey while tame animals were injected by hand. Locally fabricated, self-triggering traps (approximate size= 2m x 1m x 1m made of aluminum wire netting attached to a wooden frame), with guillotine-type trap door were used to trap free-ranging monkeys. Wild monkeys were first habituated for 2 weeks by daily placement of suitable food items around the trapping sites. Phlebotomy was via cephalic or tibial venipuncture of sedated animals. Five millilitres (5mls) of blood was collected, 1ml into heparinized tubes; the remaining into sterile plain tubes for serum separation. Samples were conveyed on ice packs to the Clinical pathology laboratory, Faculty of Veterinary Medicine, University of Ibadan where haematology and serum biochemical analyses were done immediately. Ethical approval for capture and sampling of the monkeys was obtained from the University of Ibadan, Animal Care and Use

Research Ethics Committee (UI ACUREC/App/2015/054).

Haematology and serum biochemistry

Haematology: To determine the packed cell volume (PCV) capillary tubes were filled with blood until three-quarter full. One end was then sealed with plasticine. The capillary tubes were spun in a micro haematocrit centrifuge (Hawksley and Sons, London) for five minutes at 1200×g and the PCV values were determined using the Hawksley haematocrit reader. Haemoglobin concentration was determined using the Sahli's method. Total red blood cell (RBC), total white blood cell (WBC) and platelet counts were determined manually using a Neubauer haematocytometer according to Weiss and Tvedten (2004). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were determined using specific formulae (Weiss and Tvedten, 2004). The differential WBC count was determined by identifying 100 WBCs on a blood smear to determine the percentage of each type of WBC. Total plasma protein concentration was determined using a refractometer.

Serum biochemistry: Blood was collected in plain bottles for serum preparation. Serum samples were collected by centrifugation of blood at 224g for 10 minutes. The concentrations of albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, cholesterol and glucose were determined by spectrophotometry using respective analytic kits supplied by Randox (Randox, USA). Kits were used according to manufacturer's directions. Serum globulin concentration was calculated as the difference between total serum protein and albumin concentrations

Statistical Analysis: Statistical analysis was done using SPSS 20® statistical computer software. Numerical data were summarized as mean and standard deviation of the parameters measured. Analytical statistics; Student t test and Analysis of variance, were used to compare values of blood parameters obtained between species and gender. Statistical significance was determined at 95% confidence interval.

RESULTS

Values of the haematological and serum biochemical parameters measured in 50 adult (>5years old) monkeys belonging to 4 indigenous species of both sexes are summarized in Table 2. The mean values of red cell parameters; PCV(%)/RBC($\times 10^6/\mu\text{l}$)/HB(g/dl)

Table 1: Sampling scheme for monkey species obtained in south west Nigeria

Sampling location	<i>Cercopithecus mona</i>	<i>Cercocebus Sebaeus</i>	<i>Erythrocebus patas</i>	<i>Papio anubis</i>	Total
1	2(50%) *	8(100%)	7(100%)	5(100%)	22
2	1(100%)	4(57%)	4(100%)	AV	9
3	4(100%)	NA	NA	2(100%)	6
4	4(6.7%)	NA	NA	NA	4
5	NA	2(100%)	1(100%)	1(100%)	4
6	AV	1(?)	2(?)	2(?)	5
Total	11	15	14	10	50

*Sampling proportions are in parentheses (i.e. the number of individuals sampled out of the total number of individuals of the species present in the facility); ? =unknown sampling proportion; NA= species not available at location;AV= species available at location but not sampled. 1=Zoological garden, University of Ibadan, Oyo State, 2=Biological garden, University of Ilorin, Kwara State, 3=University of Lagos, Lagos State, 4=Osun-Osogbo sacred groove, Osun State, 5=Agodi gardens, Ibadan, Oyo State, 6=Pet monkeys within Ibadan, Oyo State

Table 2: Haemogram/serum biochemical values of four species of adult (>5years) monkeys in south west Nigeria

Parameters	<i>Cercopithecus mona</i>		<i>Cercocebus sebaeus</i>		<i>Erythrocebus patas</i>		<i>Papio anubis</i>	
	Female (N=7)	Male (N=4)	Female (N=8)	Male (N=7)	Female (N=10)	Male (N=4)	Female (N=3)	Male (N=7)
Packed cell volume (%)	39.2±2*	40±3	36±4	45±4	38±3	45±4	35±3	38±8
Haemoglobin (g/dl)	11.4±4.3	13.3±1.2	11.8±1.4	14.8±1.4	12.8±1.2	14.9±1.4	11.3±1.3	12.7±2.7
Red blood cells (x10 ⁶ /µl)	6.70±0.59	6.92±0.64	5.80±0.90	7.59±0.79	6.34±0.60	7.39±0.67	5.63±0.57	6.38±1.34
MCV (fl)	58.2±2.2	57.8±0.0	62.1±2.8	59.3±0.9	59.9±1.0	60.9±0.1	62.2±1.0	59.6±0.1
MCHC (g/dl)	29.2±9.6	33.3±0.5	32.8±0.3	32.9±0.2	33.7±0.6	33.1±0.2	32.3±0.9	33.4±0.1
Platelet counts (x10 ³ /µl)	41.49±27.42	19.03±6.58	20.78±10.62	39.67±37.52	40.90±34.48	29.45±33.83	17.93±7.91	17.73±5.76
Total white cell counts/µl	7650±3660	8725±5060	6650±1858	5793±2206	5190±1502	7238±3141	5400±1146	6143±1739
Lymphocytes (%)	49±21	56±7	63±7	64±8	57±6	63±8	61±10	58±6
Neutrophils (%)	40±18	34±6	29±7	32±6	36±9	31±11	30±7	33±4
Monocytes (%)	4±1	5±2	3±2	4±2	3±1	3±1	3±1	4±1
Eosinophils (%)	7±3	6±5	3±2	2±1	3±2	4±3	6±3	5±2
Total protein (g/dl)	8.3±0.6	8.0±0.6	7.2±1.2	7.9±0.5	7.2±1.3	8.1±0.8	7.6±1.6	7.6±0.9
Albumin (g/dl)	3.5±0.2	3.3±0.6	2.9±0.5	3.1±0.4	2.8±0.8	3.5±0.4	3.4±0.6	3.0±0.4
Globulin (g/dl)	4.8±0.5	4.8±0.1	4.3±1.0	4.8±0.2	4.4±0.7	4.6±0.5	4.2±1.0	4.6±0.7
A-G Ratio	0.74±0.08	0.65±0.07	0.69±0.19	0.64±0.09	0.65±0.18	0.70±0.08	0.80±0.01	0.66±0.09
AST (IU/L)	21±4	23±4	26±6	22±5	20±3	22±5	24±5	23±4
ALT (IU/L)	28±6	32±4	32±5	30±6	27±4	32±3	32±5	32±3
ALP (IU/L)	224±77	228±91	236±67	241±58	209±48	212±70	209±70	166±55
Blood urea nitrogen (mg/dl)	12±1	12±1	11±1	11±1	11±1	11±1	12±1	11±1
Creatinine (mg/dl)	0.8±0.2	0.7±0.2	0.8±0.2	0.8±0.1	0.7±0.1	0.8±0.2	0.8±0.2	0.7±0.1
Glucose (mg/dl)	106±11	108±10	92±13	82±9	106±25	95±12	93±19	104±11
Cholesterol (mg/dl)	105±18	111±19	90±24	90±21	94±18	114±13	107±17	95±8

*All values are expressed as mean ± standard deviation. Note: PCV=Packed cell volume; RBC=Red blood cells; MCV=Mean corpuscular haemoglobin; MCHC= Mean corpuscular haemoglobin concentration; A-G Ratio=albumin-globulin ratio AST=Aspartate transferase, ALT= Alanine transferase, ALP= Alkaline phosphatase.

Table 3: A comparison of blood parameters between male and female monkeys irrespective of species differences.

Blood parameters	Sex	
	Male(N=22)	Female(N=28)
Packed cell volume (%)	41±6	37±4*
Haemoglobin(g/dl)	13.9±2.1	12.2±2.4*
Red blood cells(x10 ⁶ /µl)	7.0±1.0	6.2±0.8*
White blood cells(x10 ³ /µl)	6.7±2.9	6.2±2.4
Platelets(x10 ³ /µl)	27.1±2.6	32.8±2.7
Lymphocytes (%)	60.4±7.2	57.3±12.6
Neutrophils (%)	32.3±6.2	34.2±11.7
Monocytes (%)	3.7±1.5	3.3±1.6
Eosinophils (%)	4.1±2.8	4.4±3.1
Total protein (%)	7.8±0.7	7.5±1.2
Albumin(g/dl)	3.2±0.4	3.1±0.7
Globulin(g/dl)	4.7±0.4	4.4±0.7
A-G Ratio	0.66±0.08	0.70±0.16
AST (IU/L)	22.6±4.1	22.2±5.0
ALT(IU/L)	31.4±4.4	29.1±5.5
ALP(IU/L)	209.5±68.7	220.4±61.3
Blood urea nitrogen(g/dl)	11.2±0.8	11.2±1.0
Creatinine(g/dl)	0.77±0.14	0.76±0.16
Glucose(g/dl)	97.8±12.8	121±14.8
Cholesterol(g/dl)	99.7±18.1	97.1±19.8

*Values of corresponding parameter is significantly higher in males than in females at p≤0.05

in the males (41±6/7.0±1.0 /13.9±2.1), were significantly higher (p=0.01/ p=0.02/p=0.05) than in the females (37±4/6.2±0.8/12.0±2.4) irrespective of the species (Table 3). BUN had significant (p=0.02) variability between species with Anubis baboon and Mona monkey having a significantly higher values when compared with that from either Green or Patas monkey.

DISCUSSION

All blood parameters measured in this study appear to be similar in value across the species except for eosinophils, with relatively fewer numbers and wider ranges, which showed significant differences attributable to outlier effects. The consistently higher erythrocytic parameters in adult male monkeys relative to values in adult females is similar to findings in the Rhesus monkeys (*Mucaca mulatta*) where PCV(%) /RBC(x10⁶/µl) /HB(g/dl) values in adult males (41±4/5.5±0.4/13.2±1.3) were found to be significantly higher (p<0.01) than in adult

females ($39 \pm 3/5.1 \pm 0.39/12.3 \pm 1.3$) (Stanley and Cramer, 1966); *Cynomolgus* monkeys (*Mucaca fascicularis*) where respective values in adult males ($46 \pm 3/5.9 \pm 0.3/13.9 \pm 0.9$) were consistently higher than in adult the adult females ($44 \pm 3/5.6 \pm 0.4/13.5 \pm 0.6$) (Park et al., 2016); and in Chimpanzees where the values in adult males ($47 \pm 5/5.5 \pm 0.7/15.4 \pm 1.5$) were also consistently higher than in adult the adult females ($42 \pm 7/5.1 \pm 0.9/13.6 \pm 2.1$) (Howell et al 2003). This form of sexual dimorphism which appears to be common manifestation in all mammals has been attributed to polymorphisms of the erythropoietin (EPO) gene and its receptor (EPOR) (Zeng et al., 2001).

The white cell parameters showed no significant difference in males and females in all monkey species included in this study in agreement with findings in *Cynomolgus* monkeys (Park et al., 2016), but this is contrary to studies in other primates, such as the Rhesus monkey, in which adult males were found to have higher total white cell and lymphocyte counts but a lower neutrophil count than in adult females (Stanley and Cramer, 1966). Generally, there appears to be no uniform trend in sexual differences of white blood parameters in primates. For example, Howell et al. (2003) found that white cell parameters in male chimpanzees were not significantly different from that of the females whereas Herndon and Tigges (2001) found that total WBC and lymphocyte counts in female chimpanzees were significantly higher than in the males. Haematological values reported here for the Patas monkey (*Erythrocebus patas*) were very similar to those reported by Calle and Joslin (2014), from Composite Med ARKS Records comprising of not less than 400 individuals. Similar values were also reported for the African green monkey (Sato et al, 2005; Liddie et al, 2010) but literature is sparse for values for other monkeys sampled in this present study.

There was no significant difference between any of the serum biochemical parameters measured in male and female animals irrespective of species. The serum biochemical values obtained in this study fall within the range of values obtained in other studies on old world monkeys which typically have a relatively large standard deviation (Calle and Joslin, 2014; Castro et al., 2016). However, sexual differences were found in those studies. For example, ALT and Creatinine values for adult male obtained in the green monkey by Castro et al., (2016) was significantly higher than that of adult females while ALB and Cholesterol levels in females were higher than that of males. Inter species variations in the titre of serum biochemical parameters was generally minimal with BUN being the only parameter having significant ($p=0.02$) variability between species.

Since all animals were anesthetized for collection and examination, the effects of ketamine must be taken into consideration. Ketamine has been reported to

reduce leukocyte and red blood cell counts as well as reduce corpuscular haemoglobin and haematocrit in rhesus monkeys (*Macaca mulatta*) when blood parameters were compared between manually restrained and ketamine immobilized subjects (Swindle et al, 2002). This should therefore be taken into consideration when interpreting haematology results of monkeys using tables provided in this study.

The limited sample size did not allow for more robust comparisons of parameters across species and habitats. Despite this limitation, preliminary findings such as influence of gender on hematologic values was revealed and implies that gender peculiarities should be always be considered when evaluating haematological and serum biochemistry values for health and disease in monkey. Values reported in this study can serve as guide to developing more elaborate blood parameter reference ranges for Nigerian indigenous monkeys in the future; considering influences such as age, gender, species and captivity on physiologic status of non-human primates.

Acknowledgement: The authors are thankful to Dr (Mrs) F. Olaleru of the Department of Zoology, University of Lagos for advisory support to this work. We also appreciate the assistance of staff of the Zoological garden, University of Ibadan, Oyo state, Biological garden, University of Ilorin, Kwara state, Agodi gardens Ibadan, Oyo state and Osun Osogbo sacred groove, Osun state for their assistance during the restraint and sampling of non-human primates in their respective facilities.

REFERENCES

- Abro, A. H., Abdou, A. M. S., Gangwani, J. L., Ustadi, A. M., Younis, N. J., Hussaini, H. S. (2009). Hematological and biochemical changes in typhoid fever. *Pakistani Journal of Medical Sciences* 25 (2): 166–171
- Bhatia, R. S., Garg, R. K., Gaur, S. P. S., Kar, A. M., Shukla, R., et al. (2004). Predictive value of routine haematological and biochemical parameters on 30-day fatality in acute stroke. *Neurology India*. 52 (2): 220–223
- Calle, P. P., Joslin, J. O. (2014). New world and old world monkeys. *Zoo and Wild Animal Medicine*, eighth edition. St Louis: Elsevier Saunders, USA. pp 301–335
- Castro, J., Puente, P., Martínez, R., Hernández, A., Martínez, L., et al. (2016). Measurement of hematological and serum biochemical normal values of captive housed *Chlorocebus aethiops sabaeus* monkeys and correlation with the age. *Journal of Medical Primatology*. 45(1): 12–20. doi: 10.1111/jmp.12203
- Chichester, L., Gee, M. K., Jorgensen, M. J., Kaplan, J. R. (2015). Hematology and Clinical Chemistry Measures During and After Pregnancy and Age- and Sex-Specific Reference Intervals in African Green Monkeys (*Chlorocebus aethiops sabaeus*). *Journal*

- of American Association of Laboratory Animal Science. 54(4): 359–367
- Herndon, J. G., Tigges, J. (2001). Hematologic and blood biochemical variables of captive chimpanzees: cross-sectional and longitudinal analyses. *Comparative Medicine*. 51: 60–69
- Howell, S., Hoffman, K., Bartel, L., Schwandt, M., Morris, J., Fritz, J. (2003). Normal hematologic and serum clinical chemistry values for captive chimpanzees (*Pan troglodytes*). *Comparative Medicine*. 53: 413–423
- Kagira, J. M., Ngotho, M., Thuita, J. K., Maina, N. W., Hau, J. (2007). Hematological Changes in Vervet Monkeys (*Chlorocebus aethiops*) During Eight Months' Adaptation to Captivity. *American Journal of Primatology*. 69(9): 1053–1063
- Liddie, S., Goody, R. J, Valles, R., Lawrence, M. S. (2010). Clinical chemistry and hematology values in a Caribbean population of African green monkeys. *Journal of Medical Primatology*. 39: 389–398
- McPherson, F. J. (2013). Normal Blood Parameters, Common Diseases and Parasites Affecting Captive Non-human Primates. *Journal of Primatology*. 2(2): e112. Doi: 10.4172/2167-6801.1000112. 7
- Milner, J. M., Stien, A., Justin Irvine, R., Albon, S. D., Langvatn, R., Ropstad, E. (2003). Body condition in Svalbard reindeer and the use of blood parameters as indicators of condition and fitness. *Canadian Journal of Zoology*. 81: 1566–1578
- Park, H-U., Cho, J-W., Lee, B-S., Park, H., Han, J-S., et al. (2016). Reference values of clinical pathology parameters in cynomolgus monkeys (*Macaca fascicularis*) used in preclinical studies. *Laboratory Animal Research*. 32(2): 79–86
- Sato, A., Fairbanks, L. A., Lawson, T. P., Lawson, G. W. (2005). Effects of age and sex on hematologic and serum biochemical values of vervet monkeys (*Chlorocebus aethiops sabaues*). *Journal of American Association for Laboratory Animal science*. 44(1): 29–34
- Stanley, R. E., Cramer, M. B. (1966). Hematology of the monkey (*Mucaca mulatta*). AFRRRI Scientific report December, 1966. Armed forces Radiobiology Research Institute, Bethesda, Maryland, USA. 8pages. Available from <http://apps.dtic.mil/dtic/tr/fulltext/u2/658021.pdf>
- Swindle, M. M., Vogler G. A., Fulton, L. K. et al. (2002). Preanesthesia, anesthesia, analgesia, and euthanasia. In: Fox, J. G. Anderson, L. C. Loew, F. M. and Quimby F. W. (eds). *Laboratory animal medicine*, second edition. Academic Press, Inc., San Diego, Calif. pp. 955–1003
- Weiss, D., Tvedten, H. (2004). The complete blood count and bone marrow evaluation. In: MD Williard (ed). *Small animal clinical diagnosis by laboratory methods*, fourth edition. Elsevier, Missouri, USA, pp.15–35
- Xie, L., Xu, F., Liu, S., Ji, Y., Zhou, Q., et al. (2013). Age- and Sex-Based Hematological and Biochemical Parameters for *Macaca fascicularis*. *PLoS One*. 8(6): e64892. Available from <https://doi.org/10.1371/journal.pone.0064892>
- Zeng, S. M., Yankowitz, J., Widness, J. A., Strauss, R. G. (2001). Etiology of differences in hematocrit between males and females: sequence-based polymorphisms in erythropoietin and its receptor. *Journal of Gender Specific Medicine*. 4(1): 35–40