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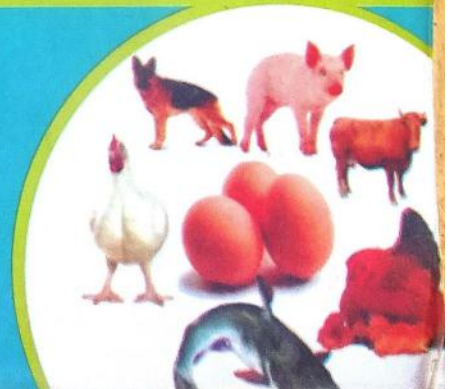
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COMPARATIVE STUDY OF HAEMOGRAM AND ERYTHROCYTE OSMOTIC FRAGILITY OF *CHLOROCEBUS SP* AND *ERYTHROCEBUS PATAS* MONKEYS

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Abstract

The present study was aimed at comparing the haemogram and osmotic fragility of *chlorocebus sp* (African green Monkeys- AGM) and *Erythrocebus Patas* (Patas Monkeys- PM). Sixteen monkeys (eight of AGM and PM) were used for the experiment. Blood samples from each monkey was collected into heparinized bottles.

Results showed that the haemogram obtained from this study was within the normal range for monkeys. However, the neutrophil/lymphocyte obtained in PM was significantly lower than the value recorded in AGM. The lower value of PCV, MCH, MCV and MCHC and the significant decreased haemoglobin in the AGM was suspected to be due to hypoxia. The result of osmotic fragility showed increase in osmotic fragility in both monkeys, however, in the AGM haemolysis was significantly higher than in the PM which may be attributed to the aboreal nature and continuous physical exertion.

Conclusion: Erythrocytes of AGM are more susceptible to fragility than PM

Keywords: Monkeys (Green, Patas), Haemogram, Osmotic fragility.

1. Introduction

The Patas monkey (*Erythrocebus patas*- PM) is one of the medium-sized primates distributed from semi-arid areas of West to Eastern Africa. It is recognized by its different names like Red or Hussard monkey which is given due to its red-brown and silver-grey morphological colour. It is also called crying monkey after the weeping sounds of its young (Galat-Luong, 1992). PM is a semi-terrestrial primate typically living in multi-female or single-male social groups composed of 75 individuals (De Jong *et al.*, 2009). PM is the only species classified under the genus *Erythrocebus*. Over 65% of PM foraging activity is carried out on the ground. They are known to be omnivorous, relying on plant material, insects, and animal material for sustenance. During the rainy season, it eats plant materials including fruits, flowers, leaves, stems and gums as well as insects, other animal material, and fungi (Nakagawa 1989; Hall 1965). According to Rowe (1996), grasshoppers, insect larvae, winged ants, leaves, flowers and fruits are main diets of PM. They spend most of their daily time for travelling and feeding. PM are a slender species colored red-brown dorsally and grey-white ventrally (Rowe 1996). The face can be recognized by a black brow ridge and nose as well as by the white area around the mouth (Loy 1974). Adult *E. p. pyrrhonotus* have a white nose, changing from black as they approach sexual maturity. During pregnancy, the facial hair of female PM noticeably lightens, potentially as a result of changing hormone levels (Palmer *et al.*, 1981). Males possess a bright blue scrotum (Groves 2001; Rowe 1996). PM exhibit a

large degree of sexual dimorphism with adult males weighing 12.4 kg (27.3 lb) and adult females weighing 6.5 kg (14.3 lb) on average (Galat-Luong et al. 1996). The PM male measures, excluding the tail, 600 to 875 mm (23.6 to 34.4 in) and the female measures 490 mm (19.3 in) on average (Rowe 1996). The maximum recorded longevity for a PM monkey in the wild is 21.6 years (Ross, 1991). Their slender bodies and long limbs are morphologically suited for terrestrial movement and speed rather than for arboreal movement (Gartlan 1974). PMs are quadrupedal and their terrestrial locomotion is extremely quick for a primate, with a maximum speed of approximately 55 km per hour (34.2 mph) reported (Hall 1968). Their especially long forelimbs facilitate this high running speed which provides an avenue of escape from predators (Etter 1973).

The green monkey (*Chlorocebus sabaues*), also known as the sabaues monkey or the callithrix monkey (Groves et al, 2005) is an Old World monkey with golden-green fur and pale hands and feet (Cawthon Lang, 2006), the tip of the tail is golden yellow as are the backs of the thighs and cheek whiskers (Cawthon Lang, 2006). They are called arboreal animals because they most of the time found climbing very high trees in the wild. It does not have a distinguishing band of fur on the brow, like other *chlorocebus* species, and males have a pale blue scrotum (Cawthon Lang, 2006). Some authorities consider this and all of the members of the genus *Chlorocebus* to be a single widespread species, *Chlorocebus aethiops*. The green monkey is a sexually dimorphic species, with males typically being slightly larger than females. Adult males weigh between 3.9 and 8.0 kg and measure between 420 and 600 mm, while the females usually weigh between 3.4 and 5.3 kg and measure between 300 and 495 mm. These monkeys are seasonal breeders, breeding during the April to June months (October and November in the Nyes area North West of Thiess), during which rainfall is heaviest. It is during these rainy seasons that fruit is most abundant.

Erythrocytes (red blood cells) have a typical shape consisting of an inner component which enables them to transport oxygen efficiently; however, they are highly susceptible to oxidative damage due to their anuclear status (Phrueksanan et al., 2014), this may in turn affect their osmotic fragility status. The erythrocyte membrane contains polyunsaturated fatty acids and redox active proteins of haemoglobin which are targets for free radical induced damage (Lutz and Bogdanova, 2013). Osmotic fragility index is a measure of the resistance of red blood cells to lysis by osmotic stress (Oyewale et al, 2011; Oyewale and Ajibade 1990). The test is generally useful to ascertain the level of stability and functionality of plasma membrane (Krogmeier et al., 1993). According to Rodak et al, (2007), Erythrocyte fragility refers to the propensity of erythrocyte (red blood cells, RBC) to haemolyse (rupture) under stress. It can be thought of as the degree or proportion of hemolysis that occurs when a sample of red blood cells are subjected to stress (typically physical stress, and most commonly osmotic and/or mechanical stress). Osmotic fragility is affected by various factors, including membrane composition and integrity as well as the cells' sizes or surface-area-to-volume ratios; (Rodak et al, 2007). Osmotic fragility is widely used to elucidate mechanisms of the influence of different factors on the osmotic properties of RBC membranes, such as shear stress and mechanical haemolysis (Sowemimo-Coker, 2002) temperature (Pribush et al, 2003). However, there is paucity of information on the erythrocyte osmotic fragility status of PM and AGM. A basic set of haematological tests can provide a wide variety of information in order to complement the osmotic fragility test. Neutrophil-lymphocyte ratio has been known to be a good biomarker of stress. The primary objective of this study was to provide a basic haematology and erythrocyte osmotic fragility of AGM and PM. This information may help the improvement of the welfare of monkeys in the area.

Under chronic stress conditions, white blood cell level becomes elevated. White blood cells are important to fight infection and healing, but if too many of them are in circulation, they

become harmful. The white blood cells are known as inflammatory leukocytes which normally are the body's defense system against infection and disease. However, when too many are produced, they contribute to a disease known as atherosclerosis. This condition is characterized by the buildup of plaque in the arteries, the major blood vessels of the body's circulatory system. The accumulation of plaque restricts blood flow, thus resulting in decreased circulation.

Materials and methods

Study Area:

The study was approved by the Ethics Committee of University of Ilorin, Ilorin Nigeria. The study was carried out at the University of Ilorin zoological garden, Ilorin, Nigeria (latitude 8.30°N and longitude 4.35°E) which is an enclosure well kept and cleaned routinely with necessary prophylaxis. The garden consists of different sections of animals including the monkeys baboons and gorilla section partitioned according to the different species. Natural features like branches and climbing platforms are being used for the enrichment of the enclosures especially for AGM, in order to imitate their natural habitat, thereby improving their welfare. The study was carried during the wet season which is usually from May to October (Ayo *et al.*, 2014).

Animals:

Sixteen apparently healthy monkeys served as subjects of the experiment. The PM and the AGM were allowed to have their food and carry out their routine grooming before commencement of the experiment. The subjects used for the study were housed at the University of Ilorin, Zoological garden, Nigeria. The PM (n=8) and AGM (n=8) were confined in standard cages and were fed twice daily and given water *ad-libitum*. Their mean body weights were 9 ± 0.96 kg for PM and 6 ± 0.4 kg, AGM. These animals are regularly dewormed in order to reduce occurrence of parasitic infections. Animal were fed twice a day with commercial monkey chew (crude protein 21.5%, and crude fat 6.8%), supplemented with fresh fruits and vegetables once a day. Animal were given free access to tap water, supplied by an automatic watering system. All experimental procedures were approved in advance by the Institutional Animal Care and Use Committee. They were allowed to feed and groom before the commencement of the experiment

Handling and blood sample collection

Each monkey was allowed to move into a cage for movement restriction and ease of capture by experienced handlers using leather- hand gloves and net according to the standard methods (Miller and Fowler, 2015). Blood samples from each monkey was collected by venipuncture from the cephalic vein into heparinized bottles; using a 5 ml syringe with 22 gauge needle for haematological analysis and osmotic fragility test. Red blood cell and white blood cell counts were evaluated using a haemocytometer (Schalm *et al.*, 1975). The packed cell volume was determined using microhaematocrit method (Baker and Silverton, 1985).

Determination of erythrocyte osmotic fragility (EOF)

Freshly collected blood from each rat into heparinized sample bottles were analyzed for erythrocyte osmotic fragility using the method described by Faulkner and King (1970) and modified by Oyewale (2011). The blood was pipetted into a set of test tubes containing phosphate buffer saline solution graded as follows- 0.1, 0.2, 0.3...0.9 g/dl of NaCl (pH 7.4), and thereafter carefully mixed and incubated for 30 minutes at room temperature ($25 \pm 2^\circ\text{C}$). The test tubes were centrifuged at 3000 rpm for 10 min using a centrifuge model IEC HN-SII (Damon/IEC Division, UK). The supernatant carefully withdrawn into a glass cuvette and the

absorbance of the supernatant read colorimetrically using Spectrometer 20 (Bausch and Lomb, USA) at a wavelength of 540 nm. The percentage haemolysis for each sample was calculated using the formula below by Faulkner and King (1970)

$$\text{percentage haemolysis} = \frac{\text{optical density of test solution}}{\text{optical density of standard solution}} \times 100$$

Statistical analysis

Results of haematological and osmotic fragility tests were expressed as mean $\pm$ standard error of the mean (Mean $\pm$ SEM) using GraphPad Prism version 5.3 for windows from GraphPad Software, San Diego, CA, USA. Result was subjected to Student's *t*-test to compare values of each species of monkeys. Differences were considered significance at $p < 0.05$.

Results

Table 1: Platelets, Total white blood cell counts and differential counts (Mean $\pm$ SEM) of monkeys (PM and AGM)

	Leucocytes ($\times 10^3/\mu\text{l}$)	Neutrophils ($\times 10^3/\mu\text{l}$)	Lymphocytes ($\times 10^3/\mu\text{l}$)	Monocytes($\times 10^3/\mu\text{l}$)	Eosinophils($\times 10^3/\mu\text{l}$)	Platelets($\times 10^6/\mu\text{l}$)
AGM	10.4 $\pm$ 1.5*	5.9 $\pm$ 0.9*	4.3 $\pm$ 0.9*	0.1 $\pm$ 0.1	0.03 $\pm$ 0.03	396.3 $\pm$ 14.7*
PM	6.4 $\pm$ 0.4†	2.6 $\pm$ 0.5†	3.8 $\pm$ 0.2†	0.2 $\pm$ 0.1	0.02 $\pm$ 0.01	370 $\pm$ 19.8†

Significant increase *, significant decrease † N=8

Significant increase observed in Neutrophil, lymphocyte and Monocyte of AGM compared with PM

Table 2: Total RBC: Red Blood Cell counts, PVC: Packed cell volume, Hb: Haemoglobin content and red cell indices (Mean $\pm$ SEM) of monkeys (PM and AGM)

	RBC($10^6/\mu\text{l}$)	PCV(%)	Hb (g/dl)	Erythrocyte indices		
				MCV(fl)	MCH(pg)	MCHC(g/dl)
AGM	5.1 $\pm$ 0.7	40.7 $\pm$ 5.8†	13.4 $\pm$ 3.0†	89.6 $\pm$ 8.7*	26.4 $\pm$ 3.3†	32.4 $\pm$ 2.9†
PM	5.0 $\pm$ 0.5	44 $\pm$ 2.0*	15.05 $\pm$ 1.6*	81.3 $\pm$ 3.7†	30.95 $\pm$ 4.4*	34 $\pm$ 2.1*

Significant increase *, significant decrease † N=8

$P < 0.05$ marks significant difference

The mean WBC count ($\times 10^3/\mu\text{l}$) are 10.4 $\pm$ 1.5 in the AGM and 6.4 $\pm$ 0.4 in PM. The mean lymphocytes value of 3.8 $\pm$ 0.2 $\times 10^3/\mu\text{l}$ in PM and 4.3 $\pm$ 0.9 $\times 10^3/\mu\text{l}$ in the AGM; and the mean Neutrophils value were 2.6 $\pm$ 0.5 and 5.9 $\pm$ 0.9 in PM and AGM respectively. Showing that all were significantly higher in the AGM than PM.

On the other hand, The mean PCV and Hb, were significantly higher in the PM than AGM. However, the increase in the RBC of PM was not significantly different from that of AGM. There was significant increase in the mean value MCH and MCHC of PM compared with that of AGM; however the mean MCV values were significantly higher in the AGM than in the PM.

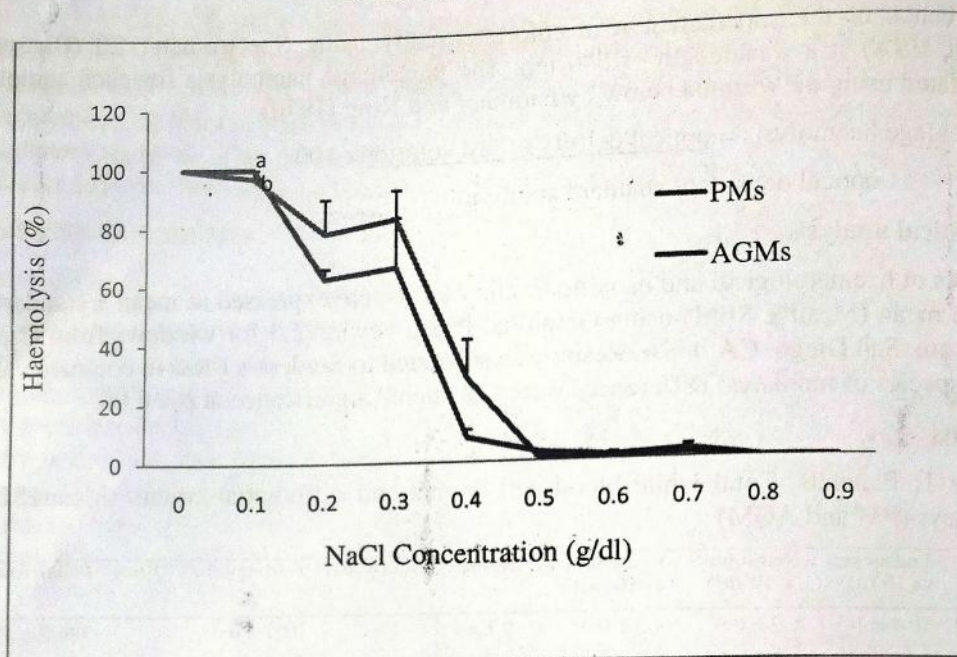


Fig 1: Comparison of basal value of Erythrocyte osmotic fragility of PM and AGM

The osmotic fragility of the red blood cells of PM and AGM were assessed and result shown as in figure 1 above. The haemolysis seen in the AGM red blood cell from 0.8g/dl concentration to 0.5 g/dl was less than 20% and insignificantly ($p>0.05$) higher than that of PM; The haemolysis in AGM was 100% at 0.1 g/dl concentration and it was significantly ($p<0.05$) higher compared with PM.

Discussion

While the PM were on the ground the AGM were on the tree and jumping from one branch to another. The haematological results obtained from this study was within the normal range for monkeys (Wang *et al.*, 2012). However, the significant increase seen in the total WBC, Neutrophils and lymphocyte obtained in AGM compared with the PM was a marker of the continuous stress in the aboreal nature of the AGM. This result agreed with the findings of Kannan *et al.* (2002) who observed an increase in neutrophil-lymphocyte ratio in goats subjected to transport stress. This however denotes that the AGM are more chronically and regularly stressed in their daily activities compared with PM. The stress may in turn elicit some immune response as confirmed by the increased neutrophil and lymphocyte counts. The lower value of PCV and RBC in the AGM does not actually mean that they are anaemic because it is still within normal value (Suqimoto *et al.*, 1986) for monkeys. However, in the indices of the red cell, MCV is significantly higher in the AGM compared with the PM, while the MCH, and MCHC recorded were lower in AGM than PM. We suspected that their staying on the tree most of the time might have subjected them to be hypoxic life in the with taller trees. in which case the erythrocyte is larger [macrocytic (MCV)] with less haemoglobin concentration. Polycythemia was not confirmed and erythropoietin was not measured however, RBC was insignificantly ($p>0.05$) higher in the AGM than PM. There is very scanty information on osmotic fragility of erythrocytes in monkeys generally. The result of osmotic fragility showed an increase in osmotic fragility in both monkeys, however, in the AGM haemolysis was significantly higher at low and high concentration (0.1 g/dl) than in the PM. Our finding is in line with the report of Rakesh and Amit, (2004) in which it was observed that Environmental heat which animals are unavoidably subjected to produce heat stress by causing discomfort, irritation and some degree of psychomotor disturbances. (The

changes in thermal environment caused by fluctuations in ambient temperature (AT) and relative humidity (RH) have been demonstrated to induce a variety of physiological responses, which may adversely affect productivity and health in the animals (Asala et al., 2011; Adenkola et al., 2010). oxidative stress produced heat which increase membrane instability of the red blood cells leading to osmotic fragility. This study has shown for the first time, the effect of stress on osmotic fragility of RBC in PM and AGM. These animals have been living their lives in the wild and now adapting to the restriction of the zoo environment could be a reason why polycythaemia was not pronounced to confirm hypoxia in the AGM. However, further studies will be carried out in other zoological gardens in this same geographical zone in the Country.

Conclusion

The lifestyle of the AGM being arboreal exposed them to continuous stress and are more stressed than the PM. The picture of the osmotic fragility showed that the RBC in the AGM will as a result of oxidative stress be subjected to lyses and short lifespan..

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Conflict of Interest

There is no conflict of interest among the authors.

References

- Adenkola AY, Ayo JO, Sackey AKB and Adelaiye AB (2010). Erythrocyte osmotic fragility of pigs administered ascorbic acid and transported by road for short-term duration during the harmattan season. *African J Biotech*,9: 226-233.
- Asala OO, Ayo JO, Rekwot PI, Minka NS, Omoniwa DO and Adenkola AY (2011). Effect of ascorbic acid administration on erythrocyte osmotic fragility of pigs transported by road during the hot-dry season. *Vet Res Com*, 35: 245-254.
- Baker FJ and Silverton RE (1985). *Introduction to Medical Laboratory Technology*. 6th Edition, Butterworth and Company Publishers Limited, U.K., 408 pp.
- Cawthon Lang K A (2006). *Primate Fact sheets: Vervet (Chlorocebus) Taxonomy, Morphology & Ecology*. Retrieved 2007-08-13.
- Connolly SD, Bernstein GA (2007) Work Group on Quality Issues. Practice parameter for the assessment and treatment of children and adolescents with anxiety disorders. *J Am Acad Child Adolesc Psychiatry*; 46(2):267-83.
- De Jong Y A Butynski, TM and Nekaris, KAI(2008). Distribution and conservation of the patasmonkey (*Erythrocebus patas*) in Kenya. *J. E. Afr. Nat. Hist.* 97: 83-102
- Etter H F (1973). Terrestrial adaptations in the hands of cercopithecinae. *Folia Primatol.* 20: 331-350
- Galat-Luong A, Bibollet-Ruche F, Pourrut X, Durand JP, Sarni P, Pichon G and Galat G (1994). Social Organisation and SIV SeroEpidemiology of a Patas Monkey Population in Senegal. *Folia Primatol.* 63:226-228

- Hall KRL (1965). Behaviour and ecology of the wildpatas monkey, *Erythrocebus patas*, in Uganda. *J.Zool.* 148:15-87
- Hall KR L (1968). Behaviour and ecology of the wildpatas monkey, *Erythrocebus patas*, in Uganda. In: Jay PC (ed). *Primates: Studies in Adaptation and Variability*. New York: Holt, Rinehart and Winston, pp 32-119
- Faulkner WR and King JW (1970). *Manual for Clinical Laboratory Procedures*, Chemical Rubber Company, Cleveland, OH, U.S.A.
- Gartlan J (1974). Adaptive aspects of social structure in *Erythrocebus patas*. In: Kondo S, Kawai M, Ehara A, Kawamura S (eds) *Proceedings from the Symposia of the Fifth Congress of the International Primatological Society*. Tokyo: Japan Science Press, pp 43-61
- Grandin T (1997). Assessment of stress during handling and transportation. *J Animal Res*, 75:249-257.
- Groves CP, Wilson DE, Reeder DM (2005). *Eds Mammals Species of the World A Taxonomic and Geographic Reference (3rd ed)*. Baltimore: Johns Hopkins University Press. 158-159.
- Kannan G, Terril TH, Kouokou B, Gelaye S, and Amoah EA (2002). Stimulated pre-slaughter holding and isolation effects on stress responses and liveweight shrinkage in meat goats. *J Animal Sci*, 80:1771-1780.
- Kingdon J (1997). *The Kingdon Guide to African Mammals*. London: Academic Press Limited.
- Kingdon J & Gippoliti S (2008). *Chlorocebus sabaeus* Red List of Threatened Species. Retrieved 4 January 2009.
- Loy J (1974). Changes in facial color associated with pregnancy in patas monkeys, *Folia Primatol.* 22:251-257
- Lutz HU and Bogdanova A (2013). Mechanisms targeting senescent red blood cells for clearance in healthy humans. *Frontiers in Physiol* 4, 387.
- Matthew Keller, (2012) "Animal Diversity Web".
- Miller RE and Fowler ME (2015). Mammal Groups, Part iv. In: Fowler, M.E. (Ed.), *Zoo and wild animal medicine: Volume 8*, Pp 360.
- Nakagawa N (1992). Distribution of affiliative behaviors among adult females within a group of wild patas monkeys in a non mating, non birth season. *Int. J. Prim.* 12: 73-96
- Oyewale JO, Dzenda T, Yaqub L, Akanbi D, Ayo J, Owoyele O, Minka N and Dare T (2011). Alterations in the osmotic fragility of camel and donkey erythrocytes caused by temperature, pH and storage. *Vet Arhiv*, 81:459-470.
- Phruksanan W, Yibchok-anun S, and Adisakwattana S (2014). Protection of *Clitoria ternatea* flower petal extract against free radical induced hemolysis and oxidative damage in canine erythrocytes. *Res Vet Sci*, 97: 358-364.
- Pribush A, Hatskelzon L, Kapelushnik J, and Meyerstein N, (2003). "Osmotic swelling and hole formation in membranes of thalassemic and spherocytic erythrocytes," *Blood Cells, Molecules, and Diseases*, 31, (1), 43-47.
- Rowe N (1996). *The pictorial guide to the living primates*. East Hampton: Pogonias Press

- Schalm OW, Jain NC and Carroll EJ (1975). *Veterinary Haematology*. Lea and Febiger, Philadelphia, Pa, U.S.A., 3rd Edition, Pp. 12-18.
- Sowemimo-Coker SO, (2002) "Red blood cell hemolysis during processing," *Transfusion Medicine Reviews*, vol. 16, (1) 46-60.
- Suqimoto Y, Hanari K, Narita H, Honjo S (1986). Normal hematologic values in the cynomolgus monkeys aged from 1 to 18 years; Jikken Dobutsu Expt animals ;35(4):443-7.
- Wang H, Niu YY, Si W, LiY J and Yan Y (2012). Reference data of clinical chemistry haematology and blood coagulation parameters in juvenile cynomolgus monkeys (*Macaca fascicularis*). *Vet Med*, 57:233-238.