ISSN: 2583-9365

Hematological and biochemical values of Common Marmoset (*Callithrix jacchus*) fed with fortified diet

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Abstract

The Common Marmoset (*Callithrix jacchus*) is the smallest non-human primate which is used frequently in biomedical research. The approximate 88-97% of similarity with human genome makes them a preferable option for use as laboratory model of human diseases and safety assessment. The present study was undertaken to provide a clinically relevant reference range for both haematology and serological parameters of *Callithrix jacchus* at the experimental animal facility of ICMR-National Institute for Research in Reproductive Health (NIRRH), which houses the largest captive-bred colony of Common Marmosets in existence. The data also gives the ambient environmental conditions in the laboratory cages and food supplement with their nutritive values to uphold the healthy colony of marmoset. We have generated an information from the values obtained in our study of 40 male and 40 female adult Common Marmoset. The hematological and biochemical values of Common Marmoset (*Callithrix jacchus*) fed with fortified diet were comparable with values reported by other researchers. These results will serve as a reference to evaluate the physiological and health status of *C. jacchus* populations, which may be useful for further research.

Key words: Common Marmoset, New World Primate, Animal Model, Hematology, captive-bred.

Abbreviations: ALT: Alanine transaminase, AST: Aspartate amino transferase, HDL: High Density Lipoprotein and LDL: low density Lipoprotein.

Introduction

Animals have been used for years in basic research and toxicity studies. The use of animal model helps to develop the fundamental knowledge necessary to understand the molecular mechanisms of normal body. It is also important to develop and establish the safety aspect of a drug. Using animals for experiments is vital as the entire complexity cannot be duplicated in cell culture or non-living system. This information can then be translated for the protection and welfare of animals and human beings. Mice and rats are the most commonly used species but there has been an increasing interest in using non-human primates for studying human disease, physiology and metabolism owing to its near-human model. The principal advantage of using nonhuman primates in research comes from their approximate 88-97% genetic similarity to humans. The average percentage of amino acid sequence conservation was observed to be 86% when 30 proteins were analysed between humans and marmoset, in contrast to 61% observed between human and mouse Kohu et al., 2008).

The Common Marmoset, *Callithrix jacchus* is a New World Monkey native to the north-eastern coast of Brazil. These primates are considered part of the *Callitrichidae* family, and are closely related to other species such as tamarins (genus *Sanguinus*) and golden lion tamarins. Males weigh 256 g on average and females weigh 236 g on average (Row, 1996). Common Marmosets (or "marmosets" unless specified otherwise in this article) have been studied in laboratories

worldwide owing to various advantages like their small size, ease of handling and fast breeding in captivity. The lifespan of marmosets is relatively short compared to other nonhuman primates (NHPs), with animals reaching maturity by 18-24 months of age, producing offspring by three years and reaching old age by eight years (Abbott and Hearn,1978). Marmosets mimic human diseases and are considered best among the non-human primate model because of its ease of handling and not known to transmit endogenous virus harmful to humans (Carrion and Patterson, 2012). Studies have found that the organization of seminiferous epithelium in the testis of marmosets is similar to that in humans and thus concluded that the marmoset provides a suitable model for studies on spermatogenesis in humans (Millar et al., 2000). Apart from the use of marmoset in neuroscience, reproductive biology, infectious diseases and behavioural research, they are widely used in drug development and toxicity studies. Marmosets have been used as a model to research various human neurological diseases like Parkinson's (Ando, et al., 2012; Eslamboli, et al., 2005), Huntington's (Kendall et al., 1998; Kendall et al., 2000), Alzheimer (Maclean, et al., 2000) and stroke (Marshall et al., 2000; Bihel et al., 2010). Literature suggests that the young age of sexual maturity, high reproduction efficiency, and similarities in placentation between humans and marmosets make them an attractive model to investigate the teratology effects of potential xenogenic compounds (Mansfield, 2003).

The aim of the present study was to provide a clinically relevant reference range for both haematology and serological parameters of *Callithrix jacchus* at the experimental animal facility of NIRRH, which houses the largest captive-bred colony of Common Marmosets in existence. These parameters are useful in the assessment and management of physiological status of marmosets. The data pertaining to the haematological values of marmoset have been first published nearly 30 years ago (Yarbrough, et al., 1984) hence we wish to add information to the recent update (Kuehnel, et al., 2012) on the reference values of Common Marmosets.

Materials and methods

1. *Animal Husbandry*

Forty adult females and forty adult male Common Marmoset bred in our colony were selected for the study. The breeding stock (12 pairs) of this colony was obtained from the Imperial Chemical Industries, Cheshire, United Kingdom in April 1978.

Animal Housing

The animals were kept in stainless steel cages and maintained under controlled temperature (23 ± 1°C) and humidity (55 ± 5%), and in a 14hr light/10hr dark cycle. Every animal was individually housed in a single cage.

Animal Feed

The monkeys were fed daily on a standard diet consisting of an apple or an orange or a sweet lime, bread, whole milk powder and bananas. Dry dates, egg and Multivitamins (A, B_{12} and D_3) were supplemented as shown in Table 1. Mebendazole (Syp. Mebex) 25mg/ kg bw was given for two days after every two months [Table 1]. Water was provided *ad lib.*

Blood Collection

Animal was restrained physically with help of marmoset restrainer at the institute. Blood samples (2 ml) were collected via femoral venepuncture from unanaesthesized animals using a 2 ml syringe and 26-gauge needle. The serum was separated by centrifugation and stored at -20^0 C until assayed. Tonoferon (East India Pharmaceuticals Ltd., India), an iron tonic was administered orally to replenish the iron levels.

Ethical information

The study was approved and ethical clearance for the use of animals in the study was obtained from the Institutional Animal Ethics Committee prior to initiation of the study and the blood collection was performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimental on Animals (CPCSEA), India.

2. *Methods of Analysis*

Hematology

The following hematological determinations were carried out in whole blood using Abacus Hematology Analyser (Diatron).

Clinical Chemistry

Serum samples was immediately separated by centrifugation of blood samples at 12000 rpm for 15 min. Serum samples were analyzed for various parameters. The leftover serum samples were stored in well labelled, tightly capped microfuge tubes at -80°C. The following parameters were analyzed using automatic biochemical and electrolyte analyzer EM 200 (ERBA Diagnostics Mannheim). Quality control standards were pre-run on the machine before analyzing our samples.

Results

The hematology values (Table 2) of both the male and females did not showed variation and were comparable to the internationally reported values. The hemoglobin of male marmoset was higher as compared to the females. These hematology values were also comparable to human values which supports the usage of marmosets as model in biomedical research. The Biochemical parameters showed (Table 3) variation amongst the female and male marmosets. The aspartate amino transferase (AST) and Alkaline Phosphatase showed more variation between the animals in both the gender. The cholesterol (174.021 ± 52.870) and triglyceride (110.246 ± 36.359) levels were well within the range and comparable with reported values. The serum calcium, Potassium, sodium, chloride and Phosphorus values did not showed high variation amongst the animals. The data were calculated as mean ± standard deviation (SD).

Discussion

This study was designed to investigate the peripheral blood parameters in Common Marmoset (*Callithrix jacchus*), which has seldom been reported. A profile that combines hematological and biochemical data is required to identify the general health of the animals also for diagnosis and treatment of diseases. However, these parameters would vary depending on their diet, housing conditions, treatment effects and various other external conditions. Hence, it is important to have a valid reference range that could help the researcher ascertain the health status of the animals.

The values obtained in our study of 40 male and 40 female healthy adult common marmoset were comparable to those obtained by other studies (Yarbrough, et al., 1984; Kuehnel, et al., 2012). We observed that the hematology values were well comparable to those of humans. Similarly, cholesterol and triglyceride levels were well within the range, substantiating that the diet provided is sufficient and healthy. The levels of serum biochemical parameters were also similar to those previously reported (Clapp, 1993). Since adequate information is unavailable for the reference of these blood parameters in marmoset, we determined them to create a reliable data set for future research. Ours is the only breeding centre and institute for research in common marmoset in India hence, the values are of utmost importance to carry out various trials.

In conclusion, monitoring the hematological and biochemical parameters can serve as a means to evaluate the physiological and health status of *C. jacchus* populations which may be useful for breeding and as an experimental model.

Acknowledgement

Authors acknowledge the assistance provided by Dr Shahir Gaikwad, Mr Sharad Bhagat, Mr. Jayant Tare, Mr. Pravin Salunkhe, Ms. Gunpriya and Mr. Subhash Kadam. Authors are thankful to Indian Council of Medical Research (RA/447/12-2016) for funding the study.

Table 1: Nutrient Supplement for marmoset

Sr. No.	Particulars	Quantity per animal	Dosage Frequency
1.	Calcium Solution	0.2 ml	Alternate day
2.	Vitamin D_3	2,500 IU	Alternate day
3.	Multivitamin Supplement Verol ™	0.25 ml	Daily
4.	Syrup Mebendazole	25 mg/kg BW	Two day after every two months

Table 2: Hematological parameters of the common marmoset

PARAMETERS	MALE n=40	FEMALE n=40
White Blood Cell ($x10^3/\mu l$)	12.796 ± 5.256	11.857 ± 5.414
Red Blood Cell ($x10^6/\mu l$)	8.256 ± 0.696	7.458 ± 1.006
Hemoglobin (g/dl)	15.361 ± 0.965	14.6136 ± 1.677
Hematocrit%	53.933 ± 4.105	46.742 ± 7.844
Mean corpuscular hemoglobin (g/dl)	28.536 ± 0.809	29.841 ± 0.952
Mean corpuscular volume (Fl)	65.500 ± 2.994	65.816 ± 2.722
Mean corpuscular hemoglobin (pg)	18.676 ± 1.091	19.659 ± 1.262
Neutrophils %	36.500 ± 28.991	51.5 ± 14.983
Lymphocytes%	42.390 ± 20.937	33.317 ± 13.167
Eosinophil %	0.500 ± 0.707	1.917 ± 0.964
Monocytes %	2.764 ± 2.288	3.245 ± 2.497

Table 3: Biochemical parameters of the common marmoset

PARAMETERS	MALE n=40	FEMALE n=19
Calcium (mg/dL)	12.984 ± 2.159	11.89 ± 20.700
Chloride (mmol/L)	108.40 ± 1.24	103.200 ± 18.888
Sodium (mmol/L)	157.375 ± 3.378	156.592 ± 4.031
Potassium (mmol/L)	3.328 ± 0.39	4.305 ± 0.573
Phosphorus (mg/dL)	5.472 ±1.853	6.922 ± 1.234
AST (U/L)	189.205 ± 88.901	125.5817±40.8055
ALT (U/L)	17.944 ± 15.614	31.34 ± 12.060
Alkaline Phosphatase (IU/L)	455.525 ± 170.624	231.5066 ± 56.1765
Albumin (g/dL)	4.681 ± 0.538	4.685±0.4486
Globulin (g/dL)	3.550 ± 0.597	3.408 ± 0.276
Albumin/Globulin ratio	1.425 ± 0.34	1.758 ± 0.691
Total Protein (g/dL)	7.804 ± 0.734	7.655 ± 0.744
Direct Bilirubin (mg/dL)	0.150 ± 0.092	0.205 ± 0.0811
Total Bilirubin (mg/dL)	0.430 ± 0.155	0.303 ± 0.176
Glucose (mg/dL)	115.938 ± 27.733	125.416 ± 36.946
Triglycerides (mg/dL)	110.246 ± 36.359	153.266 ± 42.163
Cholesterol (mg/dL)	174.021 ± 52.870	179.416 ± 45.209
HDL (mg/dL)	85.5 ± 18.358	68.040 ± 18.822
LDL (mg/dL)	81.425 ± 28.972	93.951 ± 32.320
Creatinine (mg/dL)	0.487 ± 0.191	0.471±0.061
Urea (mg/dL)	42.200 ± 15.109	31.375 ± 8.157

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