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Hematologic and Serum Biochemical Values in Aged Female Bonnet Macaques (*Macaca radiata*) Anesthetized with Ketamine Hydrochloride

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We investigated the effect of ketamine hydrochloride anesthesia on hematologic and serum biochemical values in 10 aged female bonnet macaques (*Macaca radiata*) before and 120 min after intramuscular administration of ketamine hydrochloride (15 mg/kg body weight). Ketamine anesthesia caused significant reduction in the total leukocyte count, lymphocyte count, red blood cell count, hemoglobin concentration, packed cell volume, and serum concentrations of glucose, total protein, alkaline phosphatase, calcium, sodium, and potassium. Although the effects of ketamine hydrochloride on hematologic and serum biochemical values have been reported for most of the nonhuman primates, no literature on bonnet macaques is available. These findings will be useful in designing experiments assessing pathologic and toxicologic changes in blood and serum parameters and interpreting data obtained from aged bonnet monkeys.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GLU, glucose; Hb, hemoglobin; PCV, packed cell volume; RBC, red blood cell; TP, total protein; WBC, white blood cell

Nonhuman primates, both Old World and New World monkeys, are used widely for reproductive and toxicologic studies. When used in laboratory conditions, these animals present special hazards to handlers, particularly the danger of bites and zoonotic infection.[30] Restraint or immobilization is very important to minimize the risk of harm to humans and animals when procedures like physical examination, treatment of wounds, tattooing, tuberculosis testing, and transportation are performed on animals. The most common mode of immobilization in primates is treatment with ketamine hydrochloride at an intramuscular dose of 10 to 15 mg/kg body weight.[28] Ketamine anesthesia blocks dopamine uptake and therefore elevates synaptic dopamine levels. Inhibition of central and peripheral cholinergic transmission could contribute to induction of anesthetic state and hallucinations.[1] Ketamine anesthesia not only reduces stress to the animal but also promotes safety and improves the quality of data collection. The advantages of ketamine anesthesia are its high bioavailability (93%), a broad therapeutic index, and its short-acting anesthetic effect of about 20 to 30 min.[3,18] Standardized physiologic parameters are essential prerequisites for a variety of scientific investigations.[14] Therefore precise knowledge about the effect of ketamine on hematological and biochemical parameters of laboratory animals is extremely important.

Compared with rhesus macaques, bonnet macaques are underutilized for biomedical research because of their limited availability worldwide and the fact that available published reports on hematologic and biochemical values are restricted to rhesus monkeys. Bonnet monkeys display similarities with rhesus monkeys regarding their reproductive system and anthropometric development at different stages from conception to adulthood. Unlike rhesus macaques, bonnet monkeys reproduce throughout the year in the wild and therefore are used mostly for reproductive studies.[19,23] These monkeys are abundant in the south Indian peninsula, they are smaller than rhesus monkeys, docile, easy to handle, and adaptable to captivity under laboratory conditions. Because, unlike rhesus macaques, bonnet monkeys are quite resistant to tuberculosis (unpublished observation),[17] they can be used conveniently for most biomedical research.

The resurgence of interest in aging research is phenomenal, and the availability of baseline information in aged animals may be essential for these studies. Studies in macaques for aging research have already been initiated because findings from macaques can be extrapolated directly to humans.[26] The effects of ketamine on hematology and biochemical values have been published widely for adult nonhuman primates, including the capuchin monkey,[12] the vervet monkey,[31] *Callithrix jacchus*,[27] and rhesus[2,13] and cynomolgus macaques.[11,29] However, knowledge about the normal hematologic and serum biochemical parameters of the bonnet macaque is limited,[16,21] and a direct report of the effects of ketamine hydrochloride on the blood parameters of bonnet monkeys is unavailable. To this end, we undertook the present study to determine the baseline values and effects of ketamine anesthesia on the serum hematologic and biochemical variables of aged bonnet monkeys.

Materials and Methods

Animals. This study used 10 aged female bonnet monkeys that were 18 to 20 y old and weighed 6 to 8 kg. All animals were bred and reared at the Primate Research Centre, National Institute of Immunology (New Delhi, India), and they were kept in accordance with guidelines for care and use of animals in scientific research (Indian National Science Academy, New Delhi, India)[24] in a facility registered with the Committee for the Purpose of Control and Supervision of Experimental

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Table 1. Effects of ketamine hydrochloride on hematological variables in aged female bonnet monkeys (n = 10)

	Nonanesthetized	Anesthetized	% Change
Red blood cell count ($\times 10^6$/l)	4.78 ± 0.41	4.52 ± 0.39	–5.4
Hemoglobin (g/dl)	12.41 ± 1.29	12.15 ± 1.19	–2.09[a]
Packed cell volume (%)	41.40 ± 2.56	38.4 ± 4.3	–7.2[a]
Mean corpuscular volume (fl)	86.60 ± 7.05	85.75 ± 13.50	–0.9[a]
Mean corpuscular hemoglobin (pg)	26.18 ± 4.24	27.06 ± 3.58	–3.36
Meancorpuscularhemoglobin concentration(g/dl)	30.15 ± 3.07	31.95 ± 4.93	+5.98
White blood cell count ($\times 10^3$/l)	11.21 ± 1.07	8.52 ± 0.76	–23.99[a]
Neutrophils (%)	47.8 ± 3.8	55.00 ± 3.49	+15.06[a]
Lymphocytes (%)	42.1 ± 4.1	36.5 ± 3.6	–0.13[a]
Basophils (%)	1.50 ± 0.34	1.00 ± 0.33	–33.33
Monocytes (%)	2.60 ± 0.45	1.60 ± 0.27	–38.46
Eosinophils (%)	6.00 ± 0.49	5.90 ± 0.46	–1.66

Data are presented as mean ± 1 standard deviation.
[a]$P < 0.001$.

Animals; this study was approved by the institutional animal ethics committee. The animals were maintained under standard environmental conditions (22 to 25 °C, 55% to 60% humidity, 12:12-h dark:light photoperiod) and housed individually in stainless steel nonhuman primate cages. Daily each animal was fed 100 g commercial pellet primate feed (Golden Feeds, New Delhi, India) and 50 g soaked chick peas (*Cicer arietinum*) in the morning, 2 slices of bread in the afternoon, and 350 g fruits and vegetables in the evening and had ad libitum access to water. Every 2 wk, they were supplemented with 5 ml oral B-complex vitamins (Visyneral syrup, USV, Mumbai, India) and calcium (Ostocalcium, Glaxo, Mumbai, India). All animals were tuberculosis-free, as determined by semiannual testing. The animals remained active, alert, and with no signs of clinical disorders or diseases and were not pregnant, nursing, or carrying an infant during the study period.

Blood sampling, hematologic and biochemical examinations. After overnight fasting, animals were physically restrained at the front of a squeeze cage. A hindlimb was brought forward and held firmly and the site cleaned with alcohol. A 4-ml blood sample was drawn from the saphenous vein by using a 23-gauge needle. The needle was removed from the syringe and the blood sample slowly expressed into the vial, to reduce the risk of hemolysis. A 1-ml aliquot of blood was mixed thoroughly in a vial containing ethylene diamine tetraacetic acid and used for estimating hematologic indices. For estimating various biochemical parameters, the remaining 3 ml of whole blood was allowed to clot for 1 h at room temperature and then refrigerated to retract the clot. The serum was collected within 2 h of bleeding and then stored at –20 °C for biochemical analysis, which was carried out on the following day. At 2 h after blood collection from nonanesthetized animals, the animals were anaesthetized using 15 mg/kg ketamine hydrochloride (Ketmin, Themis, Mumbai, India) intramuscularly at thigh region using 23-gauge needle. After 20 min of ketamine administration, 4 ml blood was withdrawn from the femoral vein of each of the 10 animals and processed for hematologic and biochemical examination as described.

Hematologic indices. Whole blood was analyzed for the following hematologic parameters: hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count. Hb concentration was determined using the cyanmeth method with Drabkins solution and a photoelectric calorimeter. PCV was determined by the microhematocrit method using a Hawksley (Lancing, UK) microhematocrit reader. The total WBC count was obtained with a white cell pipette and hemocytometer with a Neubauer ruler; differential leukocyte counts were determined by counting 100 cells on blood smears stained with May–Grunwald and Giemsa stain. The absolute number of each type of leukocyte was calculated from the data on the WBC and differential leukocyte counts. The RBC count was done by the Unopeptte hemocytometer method (Becton–Dickinison, Rutherford, NY). The total number of erythrocytes in 80 small (0.02 mm^2) squares of an improved Neubauer hemocytometer was recorded. Based on the above values, mean corpuscular volume was calculated as PCV × 10/RBC, mean corpuscular hemoglobin concentration as Hb × 100/PCV, and mean corpuscular hemoglobin as Hb × 10/RBC.

Serum biochemistry. Serum Na and K were estimated by the ion-selective electrode method and serum Ca by using the ortho-cresopthalein complex dye-binding micro method.[4,5] Total protein (TP), albumin, total cholesterol, glucose (GLU), blood urea nitrogen, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) were evaluated using a serum auto-analyzer (Screen Master 3000, Goa, India).

Statistical analysis. The results of hematologic and biochemical parameters obtained from blood samples collected before and after treatment with Ketamine hydrochloride were compared by using paired *t* tests (Prism, version 4.02, GraphPad Software, San Diego, CA. A P value of < 0.05 was used for statistical significance).

Results

The changes in the hematologic values of aged bonnet monkeys before and after anesthesia are shown in Table 1, and the biochemical values are shown in Table 2. Of the 10 animals under study, 7 showed decreases in RBC count, 6 animals showed decreases in Hb, and 7 animals showed decreases in PCV after ketamine anesthesia. Serum concentrations of GLU, Ca, and Na were decreased in all 10 animals, ALP and K were decreased in 9 animals, and 8 animals showed decreases in TP after ketamine hydrochloride anesthesia. The total WBC count was reduced significantly ($P < 0.001$) in 9 animals primarily due to marked reductions of circulating lymphocytes.

Discussion

Among nonhuman primates, bonnet monkeys (*Macaca radiata*) have been used as animal models for various biomedical research of reproduction, endocrinology, and disease (for example, pulmonary disease of respiratory syncytial virus, enterohemorrhagic *Escherichia coli* infection) models.[8,9,20,25] Data on the normal hematologic and serum biochemical parameters of the bonnet macaque are limited.[16,21] It has been reported

Table 2. Effects of ketamine hydrochloride on serum biochemical variables in aged female bonnet monkeys (n = 10)

	Nonanesthetized	Anesthetized	% Change
Glucose (mg/dl)	63.06 ± 9.14	50.00 ± 6.45	–20.71[c]
Blood urea nitrogen (mg/dl)	17.87 ± 4.16	18.59 ± 3.95	+4.03
Total protein (g/dl)	7.44 ± 0.57	6.93 ± 0.67	–6.85[b]
Albumin (g/dl)	3.49 ± 0.45	3.28 ± 0.51	–6.02[a]
Total cholesterol (mg/dl)	154.95 ± 16.41	153.97 ± 16.86	–0.63
AspartateaminotransferaseIU/l)	39.27 ± 13.27	38.93 ± 13.86	–0.87
Alanine aminotransferase(IU/l)	40.51 ± 13.28	40.98 ± 13.70	+1.16
Alkaline phosphatase(IU/l)	174.52 ± 60.45	170.88 ± 52.86	–2.08
Calcium (mg/dl)	10.19 ± 1.2	8.86 ± 0.69	–13.05[c]
Sodium (mmol/l)	143.3 ± 3.83	135.90 ± 3.14	–5.16[c]
Potassium (mmol/l)	4.24 ± 0.52	3.69 ± 0.37	–12.97[c]

Data are presented as mean ± 1 standard deviation.
[a]$P < 0.05$.
[b]$P < 0.01$.
[c]$P < 0.001$.

that aged female rhesus macaques have significantly higher percentages of lymphocytes, eosinophils, globulins, and uric acid and significantly lower mean corpuscular hemoglobin, total leukocyte count, low percentage of neutrophils, alkaline phosphatase and magnesium, compared with younger adult females.[10] However, that report does not describe pre- and postanesthetic values. One report on rhesus monkeys revealed that ketamine reduces hematologic parameters like the WBC count by 23% and the circulating RBC count by 8% as well as decreases serum concentrations of GLU, TP, and albumin; this reduction in WBC count is considered a result of reversal of the stress or 'alarm reaction' associated with physical restraint.[2]

In our study, ketamine anesthesia in aged bonnet monkeys caused reductions in Hb, PCV, and WBC and lymphocyte counts in addition to reductions in serum concentrations of GLU, TP, ALP, Ca, Na, and K. The decreased values of RBC and Hb are due to relaxation of the spleen and consequent splenic sequestration of erythrocytes.[7] The reduction in leukocytes was due to a decreased lymphocyte count, and the observed leukocyte reduction may have resulted because of the redistribution of WBCs from the circulation into the extravascular pool. As opposed to a relaxed state, emotional stress, excitement, apprehension, and muscular activity are known to be associated with the redistribution of lymphocytes from the extravascular pool into the circulation.[2] The decreases in concentrations of TP, albumin, and other serum analytes suggests an influx of fluid into the vascular space.[2] The decrease in GLU also may reflect the reversal of an epinephrine-induced hyperglycemia in the excited awake monkey.[2] The decrease in serum Ca, Na, and K levels in bonnet monkeys after anesthesia is supported by similar findings in rabbits as well as monkeys.[2,6,11] Ketamine relaxes various smooth muscles by reducing the intracellular Ca concentration.[22] However, no direct evidence is available to indicate the exact mechanism of action of ketamine on the serum Ca level.

There is a possibility of the stress associated with the nonanesthetized sampling affected hematologic values in the anesthetized sample. To overcome this stress effect, we provided an interval of 120 min between the nonanesthetized and anesthetized samplings. Stress-related changes in heart rate and body temperature as well as hematologic and biochemical indicators are known to be stabilized after 105 min after physical restraint in roe deer (*Capreolus capriolus*).[15] The effect of ketamine anesthesia on rhesus and cynomolgus monkeys led to a decrease in leukocyte count,[2,11] similar to findings of our present study on aged bonnet macaques. It seems that the response of the RBC count to ketamine anesthesia differs from species to species; there was decrease in RBC count in rhesus and bonnet macaques whereas increased RBC counts in cynomolgus monkeys. Serum values of creatine phosphokinase, ALT, and AST were increased in rhesus and cynomolgus monkeys,[2,11] but there was no significant increase in any of these enzymes in aged bonnet monkeys in response to ketamine treatment.

In summary, the present study shows that ketamine injection may significantly alter serum biochemical and hematologic variables in aged female bonnet macaques. These potential changes should be considered when designing studies for or interpreting data from bonnet monkeys. In addition, this study provides essential baseline information on the normal blood values of aged bonnet monkeys and the effect of ketamine on these values, which data are not readily accessible from the existing body of scientific literature on nonhuman primates.

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References

1. **Adams VHA.** 1998. The Mechanisms of action of ketamine. Anaesthes reanim **23:**60-63.
2. **Bennett JS, Gossett KA, McCarthy MP, Simpson ED.** 1992. Effect of Ketamine hydrochloride on serum biochemical and hematologic variables in rhesus monkeys. Vet Clin Pathol **21:**15–18.
3. **Clements, JA, Nimmo WS, Grant IS.** 1982. Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans. J Pharma Sci **71:**539–542.
4. **Connerty VH, Briggs RA.** 1996. Determination of serum calcium by means of Orthocresol-phthalein complexone. Am J Clin Path **45:**290-296.
5. **Dewitte K, Stockal D, Thienpont LM.** 1998. Measurement of serum sodium and potassium with direct ion-selective electrode systems. Clin Chim Acta **282:**227-228.
6. **Elsa A, Ubandawaki S.** 2005. Ketamine anaesthesia following premedication of rabbits with vitamin C. J Vet Sci **3:**239–241.
7. **Jain NC.** 1993. Essentials of veterinary hematology. Philadelphia: Lea and Febiger. p 417.
8. **Kang G, Pulimood AB, Koshi R, Hull A, Acheson D, Rajan P, Keusch GT, Mathan VI, Mathan MM.** 2001. A monkey model for enterohemorrhagic *Escherichia coli* infection. J Infect Dis **184:**206–210.
9. **Kaufman D, Smith EL, Gohil BC, Banerji M, Copalan JD, Kral JG, Rosenblum LA.** 2005. Early appearance of the metabolic syndrome in socially reared bonnet macaques. J Clin Endocrinol Metab **90:**404–408.

10. **Kessler MJ, Rawlins RG, London WT.** 1983. The hemogram, serum biochemistry, and electrolyte profile of aged rhesus monkeys (*Macaca mulatta*). J Med Primatol **12:**184–191.
11. **Kim CY, Lee HS, Han SC, Heo JD, Kwon MS, Ha CS, Han SS.** 2005. Hematological and serum biochemical values in cynomolgus monkeys anesthetized with ketamine hydrochloride. J Med Primatol **34:**96–100.
12. **Larsson MM, Birgel EH, Benesi FJ, Birgel EH Jr, Lazaretti P, Fedullo JL, Larsson CE Jr, Molina SR, Guerra PC, Prada CS.** 1999. Hematological values of *Cebus apella* anesthetized with ketamine. Braz J Vet Res Anim Sci **36:**3.
13. **Loomis MR, Henrickson RV, Anderson JH.** 1980 Effects of ketamine hydrochloride on the hemogram of rhesus monkeys (*Macaca mulatta*). Lab Anim Sci **30:**851–853.
14. **Melville GSJ, Whitecomb WH, Martinez RS.** 1967. Hematology of the *Macaca mulatta* monkey. Lab Anim Care **17:**189–198.
15. **Montane J, Marco I, Lopez-Olvera J, Perpinan D, Manteca X, Lavin S.** 2003. Effects of acepromazine on capture stress in roe deer (*Capreolus capriolus*). J Wildl Dis **39:**375–386.
16. **Mythili MD, Vyas R, Patra SS, Nair SC, Akila G, Sujatha R, Gunasekara S.** 2005. Normal hematological indices, blood chemistry and histology and ultrastructure of pancreatic islets in the wild Indian bonnet monkeys (*Macaca radiata radiata*). J Med Primatol **34:**35–40.
17. **Nagarajan P, Venkatesan R, Majumdar SS.** 2004. Unpublished observation.
18. **Ochsner AJ.** 1977. Cardiovascular and respiratory responses to ketamine hydrochloride in the rhesus monkey (*Macaca mulatta).* Lab Anim Sci **27:**69–71.
19. **O'rand MG, Widgren EE, Sivashanmugam P, Richardson RT, Hall SH, French FS, VadeVoort CA, Ramachandra SG, Ramesh V, Jagannadha Rao A.** 2004. Reversible immunocontraception in male monkeys immunized with eppin. Science **306:**1189–1190.
20. **Ponnuraj EM, Springer J, Hayward AR, Wilson H, Simoes EA.** 2003. Antibody-dependent enhancement, a possible mechanism in augmented pulmonary disease of respiratory syncytial virus in the bonnet monkey model. J Infect Dis **187:**1257–1263.
21. **Ramachandra SG, Ramesh V, Krishnamurthy HN, Ravindranath N, Shetty KT.** 1998. Normal hematological and plasma biochemical parameters of the captive bonnet monkey (*Macaca radiata*). Primates **39:**127–137.
22. **Ratz PH, Callahan PE, Lattanzio FA Jr.** 1993. Ketamine relaxes rabbit femoral arteries by reducing [Ca^{2+} sup 2+] sub i and phospholipase C activity. Eur J Pharmacol **236:**433–441.
23. **Rosario GX, Sachdeva G, Manjramker DD, Puri CP.** 2005. Enhanced expressions of endometrial tumor necrosis factor alpha and its receptors during early pregnancy in bonnet monkeys. Cytokine. Forthcoming.
24. **Sahni, SK. 2000.** Guidelines for care and use of animals in scientific research. New Delhi (India): Indian National Science Academy. p 1-26.
25. **Sandhyamani S**, **Vijayakumari A, Balraman Nair M.** 1999. Bonnet monkey model for pancreatic changes in induced malnutrition. Pancreas **18:**84–95.
26. **Shimizu Y**, **Suzuki J, Terao K, Ishida T.** 2003. In vitro aging of macaque adherent cells: similar pattern of cellular between human and macaque. Mech Aging Dev **124:**237–244.
27. **Taglioni A, Casetti AR, Bernardini A, Perretta G.** 1996. Effects of ketamine hydrochloride on haematological parameters in *Callithrix jacchus*. Folia Primatol **67:**65-68.
28. **Thurmon JC, Tranquilli WJ, Benson GJ, Lumb WV, editors.** 1996. Anesthesia of wild, exotic, and laboratory animals. In: Lumb and Jones' veterinary anesthesia. Baltimore: Lippincott Williams and Wilkins. p 727.
29. **Yoshida T, Suzuki K, Shimizu T, Cho F, Honjo S.** 1986. The effects of ketamine anesthesia hematological and serum biochemical values in female cynomolgus monkeys (*Macaca fascicularis*). Jikken Dobutsu **35:**455–461.
30. **Young SS, Schilling AM, Skens S, Ritacco G.** 1999. Short duration anesthesia with medetomidine and ketamine in cynomolgus monkeys. Lab Anim **33:**162–168.
31. **Wall HS, Worthamn C, Else JG.** 1985. Effects of ketamine anesthesia stress and repeated bleeding on the hematology of vervet monkeys. Lab Anim **19:**138–144.