



Establishment of reference values for complete blood count and blood gases in cynomolgus monkeys (*Macaca fascicularis*)

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ABSTRACT. Cynomolgus monkeys are closely related to humans phylogenetically, and this has resulted in their widespread use as a preclinical model. Hematological data with regard to these monkeys are thus important. Although reference values for blood components and sex hormones have been established for cynomolgus monkeys, those for arterial blood gases have not. The arterial blood gases quickly reflect respiratory and circulatory dynamics, and are thus useful for animal management and safe general anesthesia and surgical operations. Furthermore, since O₂ is transported by RBC, CBC and blood gases are closely related. The present study aimed to establish reference values for arterial blood gases and CBC in cynomolgus monkeys over a wide age range. Blood gases and CBC of arterial blood, collected from 41 female and 21 male anesthetized monkeys, were measured. Age correlated with RBC, HGB and HCT in the CBC. Values differed significantly between males and females in pCO₂, CO₂ concentration, MCV and MCH. The pH of blood was equivalent to that of humans and pCO₂ was more stable, whereas MCV and MCH were lower than those in humans. Erythrocytes were smaller and less pigmented than in other *Macaca* species. Several relationships between gender and age, and blood gases and CBC were identified in cynomolgus monkeys. In conclusion, these reference values will be useful as markers for veterinary applications and in the care and maintenance of these animals.

KEY WORDS: aging, arterial blood gas, complete blood count, cynomolgus monkey, nonhuman primate

J. Vet. Med. Sci.

79(5): 881–888, 2017

doi: 10.1292/jvms.16-0638

Received: 22 December 2016

Accepted: 3 March 2017

Published online in J-STAGE:

1 April 2017

Clinical science studies using experimental animal models are important for developing human therapeutics. Animals of the genus *Macaca* are primates with many physiological and anatomical similarities to humans in comparison to other common laboratory animals, such as mice. Consequently, such nonhuman primates are useful as experimental animal models. The first clinical studies to use nonhuman primates were for the development of polio vaccines in the United States and the former Soviet Union [3, 6]. In Japan, clinical studies in nonhuman primates started when the National Institute of Health established the Tsukuba Primate Research Center in 1978. Even today, clinical studies and official approval of vaccines are conducted at this center using nonhuman primates. Nonhuman primates have thus occupied an important position as an experimental animal model for human systems.

Given this background, clinical evaluation of nonhuman primates is currently conducted based on clinical data from humans and other animals. The target of this study was to establish reference values for cynomolgus monkeys (*Macaca fascicularis*). Cynomolgus monkeys are Old World monkeys of the genus *Macaca*, and are useful as an experimental animal model because of their small size and all-season breeding, which is unlike the seasonal breeding of Japanese monkeys (*M. fuscata*) and rhesus monkeys (*M. mulatta*).

Reference values for CBC, biochemical parameters, hormone parameters and electrocardiography have been established for cynomolgus monkeys [1, 2, 7, 16, 21, 23, 28]. However, the reported blood gases reference values are only for venous samples,

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and arterial blood gas values have not been reported [17]. Blood gas levels are important for advanced medical techniques' such as regenerative medicine, circulatory medicine and innovative drug development [2, 7, 9, 18]. In addition, blood gases are important as routine diagnostic parameters. Blood gases are useful for the diagnosis of cardiovascular, respiratory and metabolic diseases. Because arterial blood gases quickly reflect respiratory and circulatory dynamics, they are important for monitoring cardiovascular and respiratory diseases. Arterial blood gas data provide greater advantages than venous blood gas data. In surgical operations, monitoring of blood gases is important for safe and adequate surgical operations because they directly and accurately reflect circulation. Furthermore, examination of preoperative blood gases is vital for safe general anesthesia. From these aspects, arterial blood gas data is valuable and important. The CBC data for cynomolgus monkeys has been reported in previous studies [21, 28]. These reports describe the usefulness of CBC values, for example, in preoperative examinations and management of cardiovascular disease animals. WBC count is an effective means for deciding whether or not the animal has an infection or inflammatory disease. HCT and HGB are also correlated with the states of inflammation and dehydration [22]. The pO₂ and pCO₂ values observed in this study showed significant relationships with CBC values.

The purpose of this study was to establish reference values for arterial blood gases and CBC in cynomolgus monkeys over a wide age range. The Tsukuba Primate Research Center (National Institutes of Biomedical Innovation, Health and Nutrition) maintains approximately 1700 cynomolgus monkeys in a closed environment for biomedical research. These macaques are used for numerous research projects at this institution, including vaccine, infectious disease, gene therapy, regenerative medicine and aging studies. This colony is the largest primate experimental colony in Japan, and is used by researchers throughout the world for advanced clinical studies [2, 29, 30].

MATERIALS AND METHODS

Animals

This study used 62 cynomolgus monkeys (21 males, 41 females) bred at the Tsukuba Primate Center (Ibaraki, Japan), and was conducted according to the Rules for Animal Care and Management of Tsukuba Primate Center [10], the Guiding Principles for Animal Experiments Using Nonhuman Primates formulated by the Primate Society of Japan [11], and the Institute for Laboratory Animal Research (ILAR) Guide for Care and Use of Laboratory Animals [12]. The Animal Welfare and Animal Care Committee of the National Institutes of Biomedical Innovation Health and Nutrition (NIBIOHN, Osaka, Japan) approved the experimental protocol. All animals were individually housed in stainless-steel cages under the following conditions: temperature, 23–27°C; humidity, 50–70%; 12 air changes/hr; 12/12-hr light/dark cycle, and fed 70 g of commercial monkey chow (CMK-2; CLEA Japan, Inc., Tokyo, Japan) and 200 g of fruit daily unless otherwise indicated.

The animals had a body weight of 1.1–7.58 kg, and their age was between 1–35 years (Table 1). These represented the distribution of the animals in the facility in regards to gender and age (Fig. 1). We assigned the subject animals to the following age groups: Growth group, ≤6 years (n=9); Adult group, 7–25 years (n=43); and Aged group, >26 years (n=10). These age divisions were determined based on previous research using macaques [4, 20, 29, 30]. Additionally, the Aged group was modified to account for the lifestyle of captive cynomolgus monkeys. While cynomolgus monkeys do not generally live for longer than 26 years in the wild, this age can be achieved in captivity.

Blood sample collection

Blood was sampled from the femoral or caudal artery of monkeys under ketamine anesthesia (5–10 mg/kg IM, Ketalar; Daiichi Sankyo Propharma, Tokyo, Japan). The collected blood was immediately examined for blood gases. CBC analysis samples were collected in K2-EDTA tubes.

Analysis of blood gases and CBC

Blood gases were determined from whole blood using an autoanalyzer (Rapid Lab 348EX; Siemens, Berlin, Germany). Blood gas analysis included pH, pCO₂, pO₂, Na⁺, K⁺, Ca⁺⁺, Cl⁻, HCO₃⁻, base excess (BE), CO₂ concentration (ctCO₂), Ca⁺⁺ (7.4) and O₂SAT. CBC was determined using an automated hematology analyzer (KX-21; SYSMEX, Kobe, Japan). CBC analyses included WBC count, RBC count, hemoglobin (HGB), HCT, MCV, MCH, MCHC, platelets (PLT), red blood cell distribution width (RDW), PLT cell distribution width (PDW) and MPV.

Statistical analysis

Data were divided into age and gender groups. Statistical analyses were performed using a Student's *t*-test and Pearson's product-moment analysis. MacTK version 2.0 analysis software (ESUMI, Tokyo, Japan) was used for statistical analyses. The authors investigated blood gases and CBC parameters using the Pearson's analysis for all age groups. We also analyzed CBC and blood gas parameters using the student's *t*-test between males and females. Finally, we divided the data by age groups and examined all parameters between each group. The data are presented as arithmetic mean values ± SD. Statistical significance was set at *P*<0.05 and *P*<0.01.

Table 1. Mean age and weight in male and female cynomolgus monkeys

	n	Weight (kg) ± SD (range)	Age ± SD (range)
Male	21	5.14 ± 1.30 (2.78–7.58)	14.4 ± 9.47 (3–32)
Female	41	3.54 ± 1.10 (1.10–6.18)	17.8 ± 8.70 (1–35)

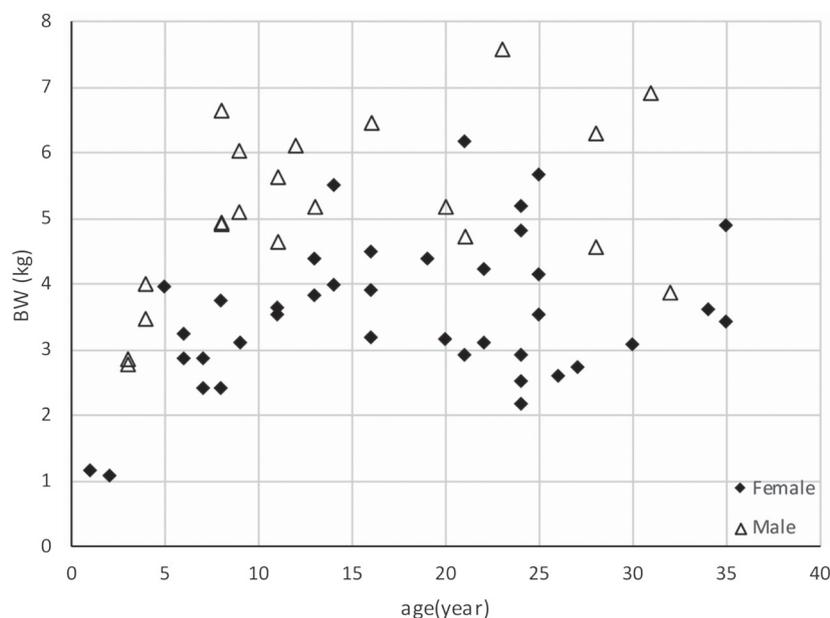


Fig. 1. Distribution of body weight and age in cynomolgus monkeys in this study. Overall, there were broad distributions of both weight and age, but weight tended to be higher in males than females.

RESULTS

Blood gases

A significant gender difference was observed for $p\text{CO}_2$ ($P < 0.01$), but there was no difference in $p\text{O}_2$ levels. Additionally, ctCO_2 differed significantly between the genders ($P < 0.05$) (Table 2). Several parameters differed between the Growth group and the other groups (Table 3). Namely, significant differences in HCO_3^- and ctCO_2 were detected between the Growth group and both Adult and Aged groups, whereas BE was significantly different between the Growth and Adult groups and $p\text{CO}_2$ was significantly different between the Growth and Aged groups (Fig. 2).

CBC

RBC, HGB and HCT were positively correlated with age (Fig. 3). MCV and MCH showed significant gender differences ($P < 0.05$). The Growth group and the other groups showed many significant differences in CBC parameters (Table 5). RBC count, HGB and HCT were significantly different between the Growth and Aged groups ($P < 0.01$). Furthermore, the Adult and Aged groups exhibited significant differences in RBC ($P < 0.01$) and HCT ($P < 0.05$).

DISCUSSION

This study is the first to report reference values for cynomolgus monkey arterial blood gases. There are some reports of blood gases of cynomolgus monkeys, but only for venous blood gases [17]. Monitoring arterial blood gases is useful for safe general anesthesia and surgical operations, which are also important from the viewpoint of animal welfare. Although reference values for CBC have already been reported for this species [21], such data have not previously been reported from large-scale experimental facilities like that in this study.

Blood gases

There was no gender specificity in blood gases (Table 2). Only $p\text{CO}_2$ and ctCO_2 showed significant gender differences; $p\text{CO}_2$ was slightly higher in males than in females. The oxidation tendencies of blood were attributed to differences between male and female cynomolgus monkeys [19, 26]. This tendency has been confirmed in humans. In rhesus monkeys, BE was reported to be

Table 2. Comparison of blood gases in male and female cynomolgus monkeys

Items	Unit	n	Mean ± SD	CI 90% ^{a)}	Male			Female			Gender Difference ^{b)}
					n	Mean ± SD	CI 90% ^{a)}	n	Mean ± SD	CI 90% ^{a)}	
pH		55	7.40 0.05	7.39–7.41	20	7.40 0.06	7.38–7.42	35	7.40 0.05	7.38–7.41	
pCO ₂	mmHg	55	36.24 4.23	35.30–37.18	20	38.57 2.89	37.45–39.69	35	34.91 4.32	33.67–36.14	**
pO ₂	mmHg	55	90.53 9.19	88.49–92.57	20	88.36 6.10	85.99–90.72	35	91.78 10.44	88.79–94.76	
Na ⁺	mmol/l	55	148.02 3.21	147.31–148.73	20	149.00 3.15	147.78–150.22	35	147.46 3.15	146.56–148.36	
K ⁺	mmol/l	55	3.70 0.40	3.61–3.79	20	3.82 0.32	3.69–3.94	35	3.64 0.43	3.52–3.76	
Cl ⁻	mmol/l	34	107.97 2.25	107.34–108.61	12	108.75 1.60	107.92–109.58	22	107.55 2.46	106.64–108.45	
Ca ⁺⁺	mmol/l	55	1.20 0.07	1.18–1.21	20	1.21 0.07	1.19–1.24	35	1.19 0.06	1.17–1.20	
HCO ₃	mmol/l	55	22.79 3.33	22.05–23.52	20	23.88 3.65	22.47–25.29	35	22.16 3.02	21.30–23.02	
BE	mmol/l	55	-2.16 4.09	-3.07–-1.25	20	-0.86 4.37	-2.55–0.84	35	-2.90 3.78	-3.98–-1.82	
ctCO ₂	mmol/l	55	23.20 3.98	22.32–24.09	20	24.88 4.02	23.32–26.43	35	22.25 3.67	21.20–23.30	*
Ca ⁺⁺ (7.4)	mmol/l	55	1.20 0.07	1.18–1.21	20	1.21 0.07	1.19–1.24	35	1.18 0.06	1.17–1.20	
AnGap	mmol/l	32	16.22 3.59	15.18–17.27	12	15.92 2.86	14.43–17.40	20	16.41 4.02	14.85–17.96	
O ₂ SAT	%	55	96.80 0.86	96.61–96.99	20	96.68 0.64	96.43–96.92	35	96.87 0.97	96.59–97.15	

a) CI 90%=90% confidence interval; b) Significant gender difference. **P*<0.05; ***P*<0.01.

Table 3. Comparison of blood gases between age groups of cynomolgus monkeys

Items	Unit	0–6 y (Growth)		7–25 y (Adult)		>26 y (Aged)		Growth vs		Adult vs	Pearson's correlation
		Mean	± SD	Mean	± SD	Mean	± SD	Adult	Aged	Aged	
pH		7.37	0.06	7.41	0.05	7.39	0.06				0.12
pCO ₂	mmHg	34.98	4.80	35.77	4.18	39.43	2.13		*	**	0.27
pO ₂	mmHg	93.64	7.55	90.20	9.66	88.80	8.87				-0.13
Na ⁺	mmol/l	147.00	2.55	148.32	3.30	147.78	3.49				0.01
K ⁺	mmol/l	3.63	0.56	3.69	0.35	3.84	0.45				0.15
Cl ⁻	mmol/l	109.25	1.89	108.17	2.10	106.33	2.50				-0.37
Ca ⁺⁺	mmol/l	1.21	0.06	1.19	0.07	1.21	0.08				0.02
HCO ₃	mmol/l	20.63	2.28	23.08	3.32	23.74	3.66	*	*		0.22
BE	mmol/l	-4.77	2.95	-1.81	4.08	-1.00	4.42	*			0.23
ctCO ₂	mmol/l	20.77	2.82	23.37	4.01	24.97	4.00	*	*		0.26
Ca ⁺⁺ (7.4)	mmol/l	1.19	0.07	1.20	0.07	1.20	0.06				0.06
AnGap	mmol/l	16.80	3.39	16.05	3.79	16.45	3.49				0.14
O ₂ SAT	%	96.86	0.87	96.84	0.89	96.60	0.83				-0.10

P*<0.05; *P*<0.01.

Table 4. Comparison of CBC parameters in male and female cynomolgus monkeys

Items	Unit	n	Mean ± SD	CI 90% ^{a)}	Male			Female			Gender Difference ^{b)}
					n	Mean ± SD	CI 90% ^{a)}	n	Mean ± SD	CI 90% ^{a)}	
WBC	×10 ² /μl	62	83.82 28.20	77.93–89.71	21	82.52 29.93	71.26–93.79	41	84.49 27.64	77.22–91.76	
RBC	×10 ⁴ /μl	62	637.24 91.06	618.22–656.26	21	613.95 85.35	581.83–646.08	41	649.17 92.59	624.82–673.52	
HGB	g/dl	62	12.48 1.65	12.13–12.82	21	12.46 1.49	11.90–13.02	41	12.49 1.74	12.03–12.95	
HCT	%	62	43.08 5.33	41.97–44.19	21	42.59 5.17	40.64–44.54	41	43.33 5.45	41.90–44.77	
MCV	fl	62	67.95 4.49	67.01–68.89	21	69.76 5.21	67.80–71.72	41	67.02 3.81	66.02–68.02	*
MCH	pg	62	19.70 1.87	19.31–20.09	21	20.43 1.75	19.77–21.09	41	19.32 1.83	18.84–19.81	*
MCHC	g/dl	62	28.97 1.68	28.62–29.32	21	29.30 1.22	28.84–29.76	41	28.80 1.86	28.32–29.29	
PLT	×10 ⁴ /μl	62	31.81 9.34	29.86–33.76	21	31.10 11.16	26.91–35.30	41	32.17 8.39	29.96–34.37	
RDW	fl	62	32.24 9.83	30.19–34.30	21	32.72 4.91	30.87–34.57	41	33.70 4.47	32.53–34.88	
PDW	fl	60	11.73 2.25	11.25–12.21	20	11.63 2.89	10.51–12.74	40	11.78 1.89	11.28–12.28	
MPV	fl	60	9.78 1.18	9.53–10.03	20	9.68 1.32	9.17–10.19	40	9.83 1.12	9.53–10.13	

a) CI 90%=90% confidence interval; b) Significant gender difference. **P*<0.05.

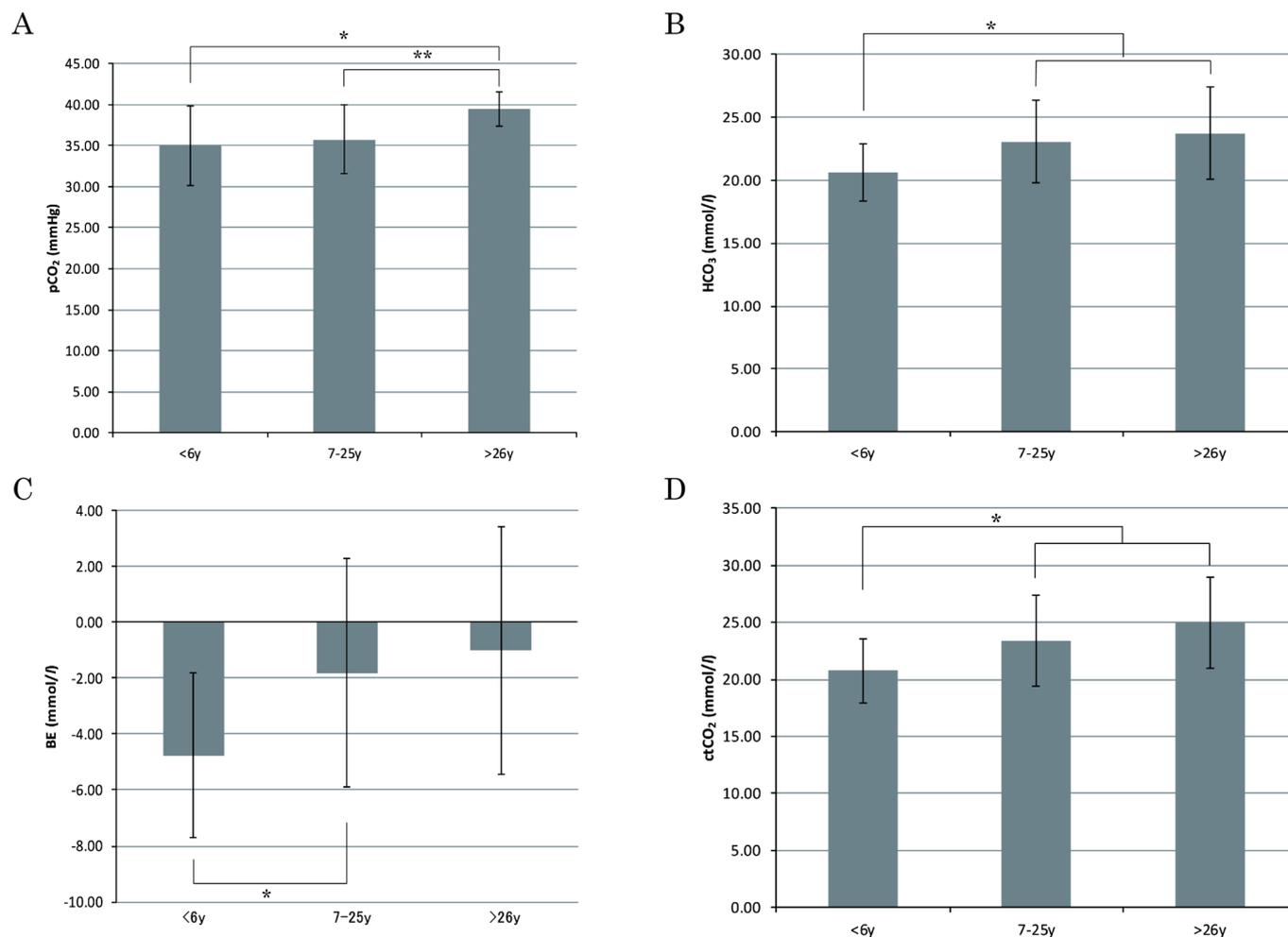


Fig. 2. Blood gas parameters plotted according to age. The pCO₂, HCO₃⁻ and BE showed significant differences. The Aged group showed an increase in pCO₂ (A), and pCO₂ increased in an age-dependent manner. The Growth group showed significant differences in ctCO₂ and HCO₃⁻ compared to the other groups (B, D). The BE increased in an age-dependent manner, and the Growth and Adult groups were significantly different from each other (C). Decreased BE indicates alkalosis (C).

lower than in humans. From these findings, the reference value for BE is approximately -3.09 mmol/l in female cynomolgus monkeys (Table 2 and Fig. 2C). Values of pH were stable and consistent with that of other primates, including humans [15]. On the other hand, rhesus monkeys show higher pH values than those of humans [9, 18]. Cynomolgus monkeys were found to show pH values closer to humans than to rhesus monkeys. In addition, pCO₂ was lower in cynomolgus monkeys than in humans, whereas pO₂ was similar to that observed in humans [15]. The pCO₂ of cynomolgus monkeys resembled that of rhesus monkeys, which suggests close values within the genus *Macaca* [18].

Significant age differences were observed for pCO₂, HCO₃⁻ and ctCO₂, which might be attributable to differences in metabolism because the circulatory system has an age-dependent decline (Fig. 2A, 2B and 2D) [14, 19]. However, this range is within the physiological value of humans. Na⁺ and K⁺ values in both genders were higher than those in humans or rhesus monkeys [15, 18], and Cl⁻ showed slightly higher values compared to those in rhesus monkeys (Table 2). Na⁺ is thought to exhibit a tendency toward high levels in the genus *Macaca*, because the levels found in rhesus monkeys were higher than in humans. Cl⁻ was shown here to be slightly higher than in humans. It should be noted that our experimental groups were composed of nutritionally controlled individuals, and external factors such as the excessive intake of salt were controlled for. Rhesus monkeys showed a similar tendency, and the genus *Macaca* are reported to show higher Na⁺ and Cl⁻ compared to humans [15, 18]. Additionally, Ca⁺⁺ was higher than in rhesus monkeys [15, 18]. The anion gap was slightly lower in cynomolgus monkeys (16.22 ± 3.59 mmol/l) compared to rhesus monkeys (17.21 ± 4.53 mmol/l), but higher than in humans (12 ± 2 mmol/l) [15]. HCO₃⁻ in the present study, as well as in previous reports for rhesus monkeys, showed a trend similar to the findings in humans [18]. Thus, the high levels of Na⁺ are thought to cause the high anion gap in genus *Macaca*. High Na⁺ can be caused by conditions such as dehydration, aldosteronism, Cushing's syndrome and other disorders; however, these conditions were not observed in our laboratory findings. The high Na⁺ level in macaques thus appears to be of physiological value. The high anion gap observed in females might be due to their low HCO₃⁻ values (Table 2). One reason for the low HCO₃⁻ is that acids carrying a negative charge, such as metabolites of

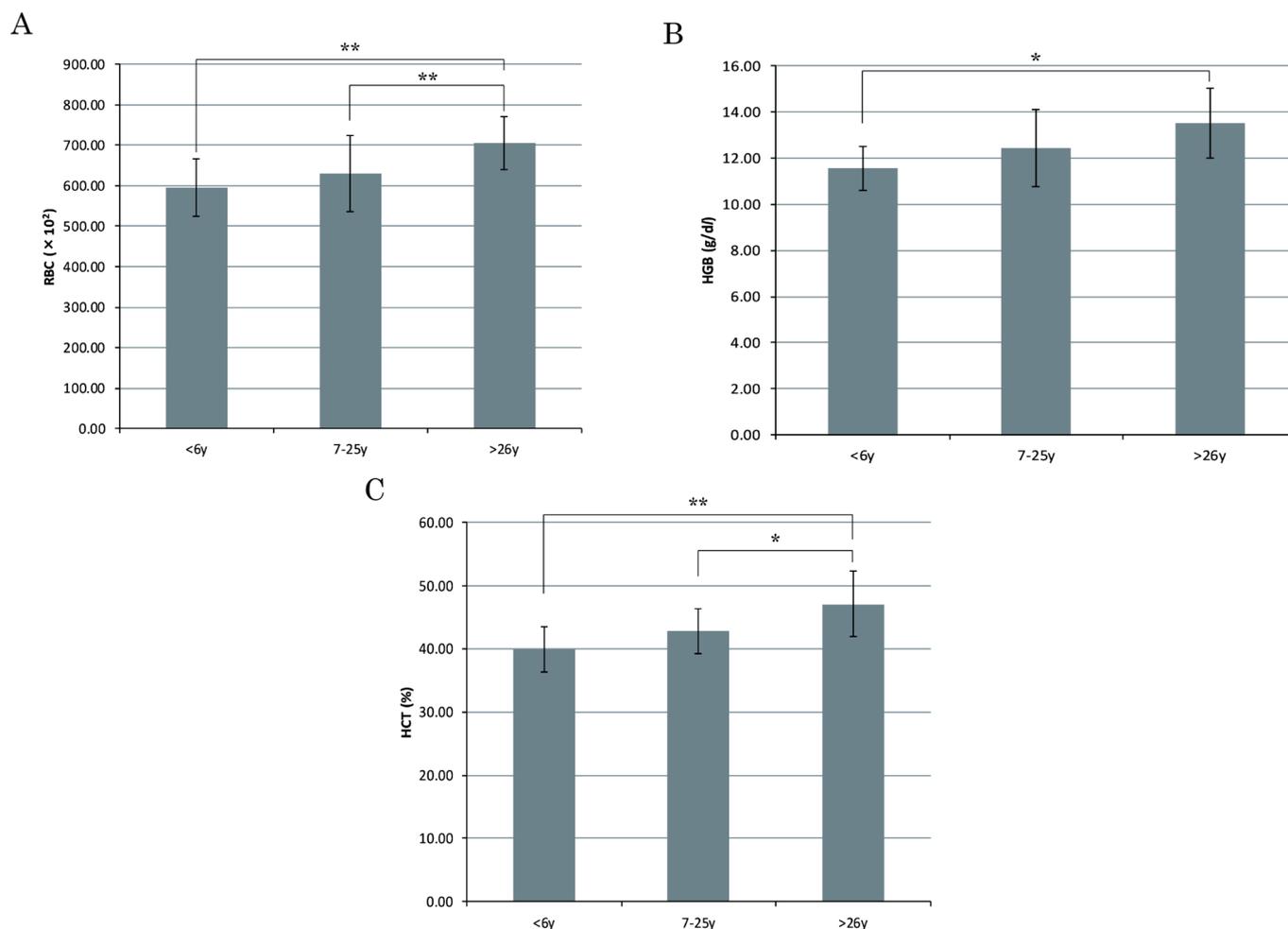


Fig. 3. Age-related changes in blood parameters. RBC count (A), HGB (B) and HCT (C) increased in an age-dependent manner, and several differences were observed between the Aged and other groups.

Table 5. Comparison of CBC parameters between age groups of cynomolgus monkeys

Items	Unit	0–6 y (Growth)		7–25 y (Adult)		>26 y (Aged)		Growth vs		Pearson's correlation
		Mean	± SD	Mean	± SD	Mean	± SD	Adult	Aged	
WBC	$\times 10^3/\mu\text{l}$	93.22	27.21	83.30	29.75	77.60	21.70			–0.06
RBC	$\times 10^4/\mu\text{l}$	595.56	72.13	630.16	92.85	705.20	65.09	**	**	0.50
HGB	g/dl	11.58	0.95	12.42	1.68	13.53	1.53	**		0.49
HCT	%	39.88	3.55	42.83	5.23	47.06	5.03	**	*	0.53
MCV	fl	67.28	4.27	68.36	4.60	66.77	4.33			–0.12
MCH	pg	19.56	1.51	19.84	1.96	19.22	1.79			–0.10
MCHC	g/dl	29.07	1.15	29.00	1.89	28.76	1.04			–0.02
PLT	$\times 10^4/\mu\text{l}$	33.49	7.55	31.87	8.58	30.02	13.82			0.04
RDW	fl	31.57	7.57	33.31	4.05	35.25	3.05			0.28
PDW	fl	11.15	1.62	11.71	2.33	12.26	2.39			0.08
MPV	fl	9.51	0.69	9.80	1.27	9.91	1.16			0.04

* $P < 0.05$; ** $P < 0.01$.

salicylic acid, ketone bodies, lactic acid, sulfuric acid, methanol and ethylene glycol, are utilized in the consumption of HCO_4^- . However, the results suggest that these conditions were not observed in the present study, and further characterization of this finding is necessary. In this study, HCO_3^- showed clear differences between the Growth and the other groups (Fig. 2B). However, significant differences were not observed in the Pearson's analysis across all age groups (Table 3). This might indicate that insufficient sample numbers were employed. This study used a large number of cynomolgus monkeys ($n=62$), and it seems that this

number is sufficient to determine standard values (Table 1 and Fig. 1). However, there is a need for validation at other institutes in the future.

CBC

Comparing the CBC values of this study with those of previous reports for cynomolgus monkeys, similar results were evident for many parameters such as RBC count, HGB, HCT, MCV, MCH and MCHC [21].

In this study, RBC count, HGB and HCT were slightly higher in females than in males (Table 4). In general, these low values in females have been attributed to menstruation [15, 21]. The high values for female RBC count in this study were attributed to less variation in the age of males compared to females. This is characteristic of many breeding colonies, where the goal is to achieve large litter sizes. Our study did not show significant differences between genders in RBC, HGB and HCT. The use of larger sample numbers is expected to attenuate these differences. MCV and MCH were lower than in humans. In reports of rhesus monkeys, MCV was lower than in humans. Therefore, all *Macaca* RBC levels are thought to be lower than that in humans [15]. MCH has been reported to show significantly lower values in cynomolgus monkeys than in humans, and a low value compared with rhesus monkeys [9, 18, 24, 25]. RBCs in cynomolgus monkeys thus appear to be less pigmented (Table 5). RBCs become more pigmented with aging. A similar phenomenon can be seen in human blood analysis. The parameters of RBC count, HGB and HCT increased in an age-dependent manner (Fig. 3). Similar results have also been observed in other monkeys [5, 13, 22, 27].

Relationship between blood gases and CBC

Blood gas and CBC values are related to the circulatory system, which is closely related to aging, dehydration, and inflammatory diseases [8, 22]. Since O₂ is carried by RBC, pO₂ is closely related to RBC count, and declines in these values are important factors in assessing physiological functions, such as erythropoietin production in the kidney.

There is no aging effect on pO₂ (Table 3). However, HCT, HGB and RGB (which are related to pO₂) increase with age (Fig. 3). In humans, these values decrease with age [8]. Previous research showed that the RBC value increased in aged macaques, suggesting a relationship with maintenance of pO₂ [5, 13, 22]. On the other hand, CO₂ is carried by serum rather than RBC. It is already known that in cynomolgus monkeys circulatory function decreases with age. Therefore, pCO₂ responds to circulatory dynamics and increases with age (Fig. 2A) [14].

The measurements obtained in this study should prove useful as reference values for blood gases and CBC parameters in cynomolgus monkeys over a wide range of ages. These reference values will be useful as markers for veterinary applications and in the care and maintenance of these animals.

ACKNOWLEDGMENTS. We wish to thank Hiromi Ogawa for the handling and care of monkeys. We are grateful to Keiko Ohto and Mayuko Tanaka for the excellent technical assistance. This work was supported by JSPS KAKENHI Grant Numbers 17790515, 24615009, and 15K07789, as well as grants from the Japan Agency for Medical Research and Development.

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