



Reference values of hematology, biochemistry, and blood type in cynomolgus monkeys from cambodia origin

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Cynomolgus monkeys as nonhuman primates are valuable animal models because they have a high level of human gene homology. There are many reference values for hematology and biochemistry of Cynomolgus monkeys that are needed for proper clinical diagnosis and biomedical research conduct. The body weight information and blood type are also key success factors in allogeneic or xenogeneic models. Moreover, the biological parameters could be different according to the origin of the Cynomolgus monkey. However, there are limited references provided, especially of Cambodia origin. In this study, we measured average body weight of 2,518 Cynomolgus monkeys and analyzed hematology and serum biochemistry using 119 males, and determined blood types in 642 monkeys with Cambodia origin. The average body weight of male Cynomolgus monkeys were 2.56 ± 0.345 kg and female group was 2.43 ± 0.330 kg at the age from 2 to 3 years. The male group showed relatively sharp increased average body weight from the 3 to 4 age period compared to the female group. In hematology and biochemistry, it was found that most of the data was similar when compared to other references even though some results showed differences. The ABO blood type result showed that type A, B, AB, and O was approximately 15.6, 33.3, 44.2, and 6.9%, respectively. The main blood type in this facility was B and AB. These biological background references of Cambodia origin could be used to provide important information to researchers who are using them in their biomedical research.

Keywords: Cynomolgus monkey, cambodia origin, body weight, hematology, serum biochemistry, blood type

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Nonhuman primates are excellent models for studying biomedical research and have been used for many decades because of their genetic similarity to humans [1]. The phylogenetic closeness between human and nonhuman primates indicates that they have intertwined with many specific genetic mechanisms related to disease [2]. Moreover, gene maps of nonhuman primates, especially Old World species, are highly conserved similar to humans [3]. These advantages of nonhuman primates fueled the study in biomedical research field to

control various human physiological processes. In non-clinical studies, the nonhuman primate results have been credited as the most reliable data when compared to other animal models (rodent, canine, and porcine) in anatomy, physiology and endocrinology [4].

Among primates, Cynomolgus monkeys (*Macaca fascicularis*) have been widely used in biomedical studies (toxicology, pharmacology, immunity, hematopoiesis, drug efficiency, infectious disease, transplantation and metabolic behavior) due to their well defined biology

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[5]. Generally, the life span of a Cynomolgus monkey is 25-30 years. The monkeys aging 19-31 months are considered juveniles with no sexual dimorphism and those of 32-44 months are considered adolescent (not yet sexually mature) [6]. Their sexual maturity is achieved in four years of age for females and six years for males [7]. They have age-related pathologies which are commonly observed in humans, such as cardiovascular disease, Alzheimer's disease [8,9], bone loss [10], knee osteoarthritis [11], obesity, diabetes and diabetic complications [12]. The Cynomolgus monkeys are also widely used as animal models for allo- or xeno-transplantation. Although there are published studies about hematology and biochemistry values of Cynomolgus monkeys, the sample size is relatively small [13,14]. Therefore, as the use of Cynomolgus monkey increase, there needs to be reliable reference values for hematology and biochemistry parameters for precise clinical diagnosis and proper interpretation of data after conducting biomedical research [15]. Additionally, the body weight and blood type information are also important factors in allogeneic or xenogeneic model studies.

The Cynomolgus monkey can be found in the tropical insular of Southeast Asia regions (main habitat; such as Cambodia, Vietnam, Philippines and Indonesia) and island of Mauritius [16,17]. The biological reference values could also be influenced by the geographical origin. Asian macaques found in Mauritius, for instance, have begun to show some isolation effect when compared to those in Asia, including a low major histocompatibility complex (MHC) diversity and genetic homogeneity [17]. In South Korea, Cynomolgus monkeys for biomedical research have been imported mostly from China which is not its native habitat. The Chinese Cynomolgus monkeys are of unknown origin, most of which probably originated from Indochina, and most monkeys originating in different countries are typically interbred in Chinese breeding farms [18]. From 2013, the Cynomolgus monkeys from Cambodia (mainland Southeast Asia) were introduced to Korean researchers by Orient Cam Co., Ltd. (Orient Cam), through importing international standard quality monkeys from their breeding facility set up in Cambodia. However, there are limited background references for Cynomolgus monkeys, especially for Cambodia origin. The Study here will add some data to the limited background references (body weight, hematology, serum chemistry, and blood types) for Cynomolgus monkeys, especially of Cambodia origin.

In this study, therefore, we performed measurements of average body weight of the Cynomolgus monkey according to sex and age, and analyzed hematology and serum biochemistry. Lastly, the blood type of the Cynomolgus monkeys was determined. Since this is the first time using Cynomolgus monkeys of Cambodia origin for biomedical research in Korea, we believe researchers could benefit from this study when using Cambodia origin Cynomolgus monkeys.

Materials and Methods

Animals

All experimental subjects were Cynomolgus monkeys from the breeding facility of Orient Cam in Cambodia. The measurements of body weight and determination of blood type were performed in this facility. The hematology and serum biochemistry were performed at the laboratory of Genia Inc. (Genia; Seongnam, Korea) in accordance with standard operation procedures. All procedures were in compliance with Animal Welfare Act Regulations and the Guide for the Care and Use of Laboratory Animals. All experiments were performed after approval by the Institutional Animal Care and Use Committee (IACUC) of Orient (ORIENT-IACUC-14195). The breeding facility has been audited by Korean Quarantine Inspection Agency and Japan Ministry of Agriculture, Fishery and Forest, and was accredited having an acceptable operation status.

All animals were housed in the primate breeding facility and had a quarantine period of at least 40 days prior to being transported to Korea. They were tested for Herpes B virus, Simian Retrovirus (SRV) and Simian T-cell Lymphotropic virus (STLV). They were also tested for tuberculosis and their feces were examined under microscope for parasites. The acclimatization period was set 6 weeks and with at least 3 weeks kept to the same housing conditions.

After finishing quarantine, all Cynomolgus monkeys were housed, bred, and cared for at a monkey facility in Genia. The environmental conditions were maintained and recorded by a central computer-assisted system with temperature at $24\pm 4^{\circ}\text{C}$, relative humidity at $50\pm 20\%$, 10-15 air-changes/h, and artificial lighting approximately 300 lux, 12 h light/dark cycle. The animals had free access to food (PMI Lab diet 5048 and fresh vegetables or fruits) and tap water which is sterilized by RO and UV. All the above environmental conditions as well as

all the procedures of housing and handling of experimental animals were in strict compliance with those conditions established by the Korean Food and Drug Administration (KFDA).

Measurement of Body Weight

The body weight data of *Cynomolgus* monkeys was accumulated in a span of a year by Orient Cam in Cambodia for their psychological monitoring purposes. The number of the subjects was 2,518 (588 males and 1,930 females). To sedate the monkeys, 1.4-4.5 mg/kg of Zoletil 50 was administered by intramuscular (IM) according to SOP code no. VET 23.1 "Sedation and restrain animal". Each body weight was measured after reset calibration in order to ensure the accuracy and reliability of the measurement results. The values of the body weight were indicated as mean and standard deviation in accordance with their different age ranges. The differences in sex were compared based on the average body weight of both sexes.

Hematology and serum biochemistry

To analyze hematology and serum biochemistry, 119 male *Cynomolgus* monkeys (19-28 months; $n=59$ and 39-72 months; $n=60$) were used. Conscious animals were physically restrained by experienced animal technicians for blood collection during quarantine period. Approximately 2.5 mL of blood was obtained by disposable syringe via femoral venipuncture and transferred into 0.5 mL EDTA tubes for hematological determinations. For serum biochemistry determinations, 2 mL of whole blood was collected in serum separator tube (SST) for serum separation. Hematology samples were processed within 1 hour after collection. Samples for serum biochemistry were allowed to clot at room temperature for 15 min and they were centrifuged at 3,000 rpm for 15 min at 4°C. The separated serum was assessed for the presence of hemolysis. All the above procedures were performed by the same technical staff to avoid variations in handling and blood drawing from animals.

Hematology values were determined by using CELL-DYN[®] 3700 hematologic analyzer (Abbott Diagnostics, Wiesbaden, Germany) and serum biochemistry was evaluated using Hitachi clinical analyzer 7180 (Hitachi Ltd., Tokyo, Japan) in Genia. Hematology analysis included white blood cell (WBC; $10^3/\mu\text{L}$), neutrophil (N; $10^3/\mu\text{L}$ and percentage), lymphocyte (L; $10^3/\mu\text{L}$ and

percentage), monocyte (M; $10^3/\mu\text{L}$ and percentage), eosinophil (E; $10^3/\mu\text{L}$ and percentage), basophil (B; $10^3/\mu\text{L}$ and percentage), red blood cell (RBC; $10^6/\mu\text{L}$), hemoglobin (Hb; g/dL), hematocrit (Hct; percentage), mean corpuscular volume (MCV; fl), mean corpuscular hemoglobin (MCH; pg), mean corpuscular hemoglobin concentration (MCHC; $10^3/\mu\text{L}$ and percentage), and platelet count (PLT; $10^3/\mu\text{L}$). Biochemistry analysis was included total protein (TP; g/dL), albumin (ALB; g/dL), globulin (GLB; g/dL), albumin/globulin ratio (A/G ratio), glucose (GLU; mg/dL), alanine aminotransferase (ALT; IU/L), aspartate aminotransferase (AST; IU/L), total bilirubin (T-BIL; mg/dL), urea nitrogen (BUN; mg/dL), creatinine (CREA; mg/dL), cholesterol (CHOL; mg/dL), triglyceride (TG; mg/dL), calcium (Ca; mg/dL), phosphorus (P; mg/dL), sodium (Na; mEq/L), potassium (K; mEq/L), and chloride (Cl; mEq/L).

Determination of Blood Type

Human blood samples were obtained from healthy men who had blood type A or B. The whole blood (10 mL) was collected in EDTA tube and then it was shaken well enough to avoid blood coagulation. In order to make 1 mL of 10% RBC suspension, 0.3 mL of human blood and 2 mL of 0.9% saline were mixed in a test tube and slowly rotated to avoiding hemolysis. After this procedure, it was centrifuged at 3,400 rpm for 2 min and then the supernatant was removed. This process had been repeated until the supernatant became clear. Finally, 0.1 mL of the packed cell (pure RBC) and 0.9 mL of 0.9% saline was mixed to make a 10% RBC suspension.

In collecting *Cynomolgus* monkey blood, all 642 numbers of the monkeys' ID numbers were verified. The blood was put in a SST and then was mixed with clot activator in order to obtain the serum. The whole blood was centrifuged at 3,000 rpm for 15 min after blood agglutination had been observed in SST. Two drops of *Cynomolgus* monkey serum were separately placed on two new places on Acryl plate. Then one drop of 10% RBC suspension obtained from Human blood type A or B was mixed with each serum which had been already dropped. The agglutination of blood was observed at 10 sec after being rolled on Acryl plate and determined the blood type of *Cynomolgus* monkey.

Results

Measurement of Body Weights

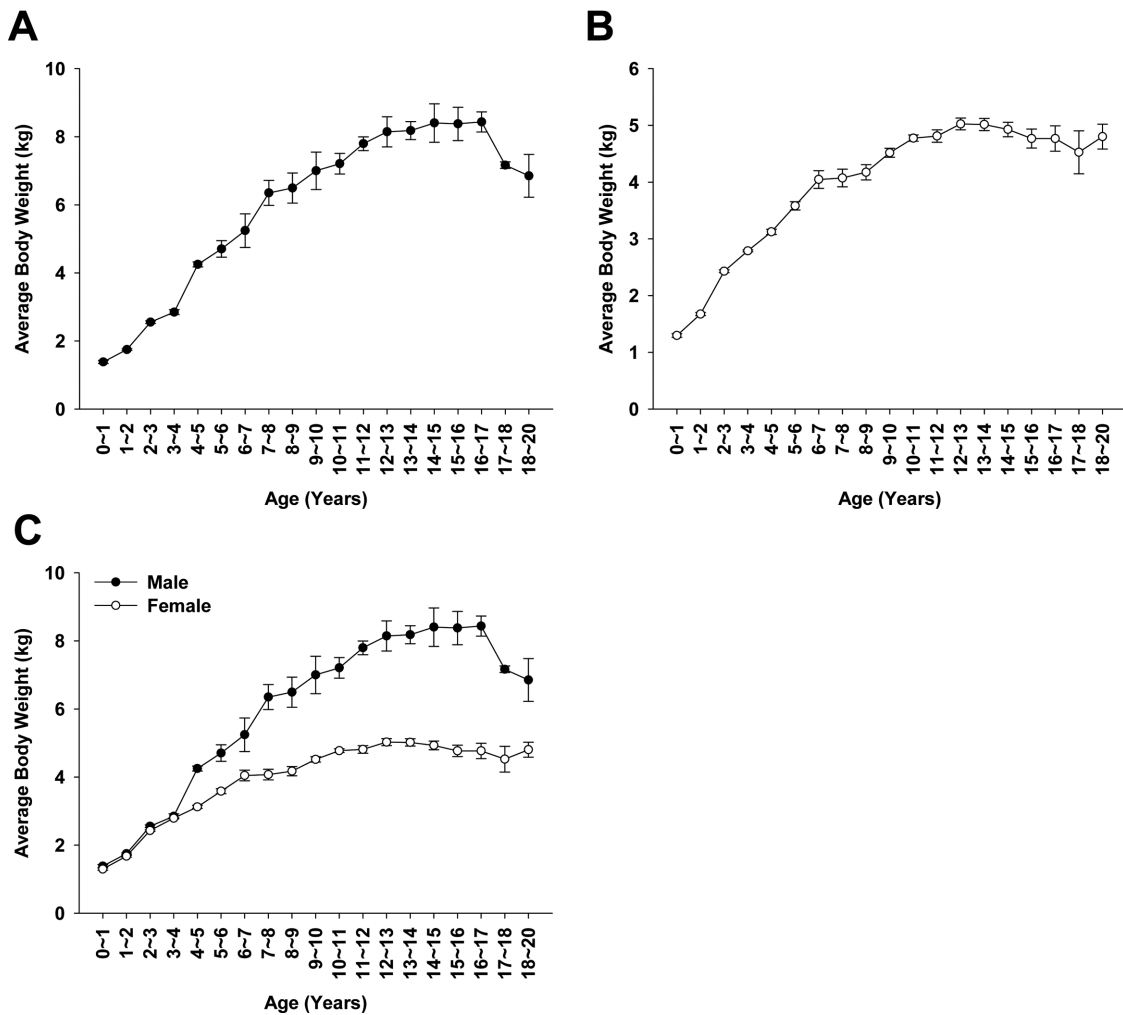


Figure 1. Average body weight of Cynomolgus monkeys. 588 male Cynomolgus monkeys (A) and 1,930 female Cynomolgus monkeys (B) were measured body weight by Orient Cam in Cambodia. Compare of average body weight between male (●) and female (○) Cynomolgus monkeys (C). All graphs were represented as mean±S.E.M.

The body weights of 2,518 Cynomolgus monkeys were measured at the breeding facility of Orient Cam in Cambodia, in a span of a year for their psychological monitoring. Both sexes of Cynomolgus monkeys were used as subjects; the male group (588 monkeys, from 10 months to 19 years old) and a female group (1,930 monkeys, from 9 months to 19 years old). The average body weight of the male group dramatically increased among the age of 7~8 years old with the average body weight being 6.35 kg (Figure 1A). However, beyond this age, the body weight increase rate was reduced and no significant change was observed from ages 12 to 17. Moreover, Cynomolgus monkeys aged over 17 showed gradual decrease in their average body weight. In female groups, the average body weight increased annually until the age of 7 (4.04 kg), but the increasing rate also

showed decline beyond this age which had similar pattern observed from male Cynomolgus monkeys and their body weight was reduced from 14 years of age (Figure 1B). When comparing the average body weight of both groups, ages from 3 to 4 years old, male Cynomolgus monkeys significantly showed increase in average body weight and the difference between male and female groups was larger (Figure 1C). The maximum average body weight in the male group was 8.43 kg, reached at the age of 16 to 17 year whereas the female group reached the peak earlier, 5.02 kg, at the age group of 12 to 13 years old. Moreover, individual variations of females were relatively lower than those of male group. The summarized result of average body weights in accordance to age and sex was represented in Table 1.

Table 1. Average body weights of Cynomolgus monkey according to age and sex

Age (year)	Males		Females	
	Animal No. (n=588)	MEAN±SD	Animal No. (n=1,930)	MEAN±SD
0-1	20	1.38±0.205	24	1.30±0.161
1-2	119	1.75±0.260	99	1.67±0.271
2-3	80	2.56±0.345	162	2.43±0.330
3-4	112	2.85±0.756	404	2.79±0.360
4-5	73	4.25±0.630	191	3.12±0.632
5-6	4	4.70±0.481	112	3.58±0.778
6-7	6	5.24±1.206	26	4.04±0.795
7-8	10	6.35±1.160	18	4.07±0.664
8-9	8	6.49±1.253	54	4.17±0.991
9-10	6	7.00±1.344	136	4.51±0.931
10-11	19	7.20±1.315	278	4.77±0.918
11-12	12	7.79±0.701	82	4.81±0.994
12-13	19	8.14±1.925	82	5.02±0.939
13-14	51	8.18±1.878	111	5.01±1.129
14-15	20	8.40±2.523	65	4.93±1.028
15-16	19	8.38±2.132	43	4.77±1.098
16-17	4	8.43±0.591	32	4.77±1.265
17-18	2	7.16±0.131	7	4.52±1.002
18-20	4	6.85±1.258	4	4.80±0.439

Table 2. Summary of Hematology in Cynomolgus monkeys of Cambodia origin

Items	Abbreviation	Units	Mean±SD		
			19-28 months (n=59)	39-72 months (n=60)	Total (n=119)
Body Weight	BW	kg	2.1±0.18	4.4±0.83	3.2±1.32
White Blood Cell	WBC	x10 ³ /μL	10.0±3.68	10.5±4.10	10.3±3.89
Neutrophils	N	%	48.9±16.14	52.7±14.46	50.8±15.37
Neutrophils	N	x10 ³ /μL	4.9±2.82	5.7±3.50	5.3±3.19
Lymphocytes	L	%	42.8±15.20	38.6±13.91	40.6±14.65
Lymphocytes	L	x10 ³ /μL	4.2±2.18	3.9±1.69	4.1±1.95
Monocytes	M	%	6.4±3.87	6.7±2.55	6.6±3.26
Monocytes	M	x10 ³ /μL	0.6±0.41	0.7±0.38	0.7±0.40
Eosinophils	E	%	1.2±1.17	1.4±1.34	1.3±1.26
Eosinophils	E	x10 ³ /μL	0.1±0.15	0.2±0.14	0.1±0.15
Basophils	B	%	0.7±0.55	0.5±0.39	0.6±0.48
Basophils	B	x10 ³ /μL	0.1±0.05	0.1±0.04	0.1±0.05
Red Blood Cell	RBC	x10 ⁶ /μL	5.4±0.37	5.5±0.50	5.5±0.44
Hemoglobin	Hb	g/dL	13.1±0.78	13.2±1.15	13.2±0.98
Hematocrit	Hct	%	37.1±5.03	38.8±3.91	38.0±4.56
Mean Corpuscular Volume	MCV	fl	68.2±7.67	71.1±6.75	69.6±7.33
Mean Corpuscular Hemoglobin	MCH	pg	24.1±0.96	24.3±1.82	24.2±1.45
Mean Corpuscular Hemoglobin Concentration	MCHC	%/g/dL	35.8±4.14	34.4±3.43	35.1±3.85
Platelet Count	PLT	x10 ³ /μL	376.5±136.12	408.1±98.20	392.4±119.08

Hematology and Serum Biochemistry

The younger male Cynomolgus monkeys (19-28 months old) and older male Cynomolgus monkeys (39-72 months old) were compared in hematology and serum biochemistry. Hematology values are summarized in Table 2. Most of the hematology values resulted had

similar results between younger and older groups. However, the values of neutrophils, lymphocytes, and platelet had big differences among individuals. Table 3 is summarized average hematology values with other references (KFDA, Mauritius origin, China origin). The hematology values in Mauritius Cynomolgus monkeys

Table 3. Hematology values comparison with other references

Items	Units	KFDA	Mauritius	China	Cambodia
White Blood Cell	$\times 10^3/\mu\text{L}$	6.1-12.5	7.0 \pm 1.77	11.74 \pm 2.50	10.3 \pm 3.89
Neutrophils	%	35-61	25.8 \pm 14.26	32.31 \pm 8.70	50.8 \pm 15.37
Neutrophils	$\times 10^3/\mu\text{L}$	-	1.91 \pm 1.60	-	5.3 \pm 3.19
Lymphocytes	%	34-56	68.3 \pm 14.45	61.91 \pm 8.08	40.6 \pm 14.65
Lymphocytes	$\times 10^3/\mu\text{L}$	-	4.66 \pm 1.116	-	4.1 \pm 1.95
Monocytes	%	0.4-3.0	4.3 \pm 1.29	3.23 \pm 0.75	6.6 \pm 3.26
Monocytes	$\times 10^3/\mu\text{L}$	-	0.31 \pm 0.129	-	0.7 \pm 0.40
Eosinophils	%	1.3-9.1	0.6 \pm 0.42	0.75 \pm 0.43	1.3 \pm 1.26
Eosinophils	$\times 10^3/\mu\text{L}$	-	0.04 \pm 0.034	-	0.1 \pm 0.15
Basophils	%	0.0-0.2	0.3 \pm 0.15	2.51 \pm 1.38	0.6 \pm 0.48
Basophils	$\times 10^3/\mu\text{L}$	-	0.02 \pm 0.011	-	0.1 \pm 0.05
Red Blood Cell	$\times 10^6/\mu\text{L}$	5.3-6.3	7.21 \pm 0.48	5.47 \pm 0.03	5.5 \pm 0.44
Hemoglobin	g/dL	11.0-12.4	14.3 \pm 0.49	13.27 \pm 0.66	13.2 \pm 0.98
Hematocrit	%	33.1-37.5	50.2 \pm 2.39	48.27 \pm 2.29	38.0 \pm 4.56
Mean Corpuscular Volume	fl	59-66	70 \pm 3.3	88.32 \pm 3.14	69.6 \pm 7.33
Mean Corpuscular Hemoglobin	pg	19-21	19.9 \pm 0.82	24.27 \pm 0.71	24.2 \pm 1.45
Mean Corpuscular Hemoglobin Concentration	%/g/dL	32-35	28.6 \pm 0.94	27.53 \pm 0.91	35.1 \pm 3.85
Platelet Count	$\times 10^3/\mu\text{L}$	300-512	373 \pm 78.9	404.5 \pm 68.9	392.4 \pm 119.08

Table 4. Summary of serum biochemistry in Cynomolgus monkeys of Cambodia origin

Items	Abbreviation	Units	Mean \pm SD		
			19-28 months (n=59)	39-72 months (n=60)	Total (n=119)
Body Weight	BW	kg	2.1 \pm 0.18	4.4 \pm 0.83	3.2 \pm 1.32
Total Protein	TP	g/dL	7.2 \pm 0.44	7.7 \pm 0.58	7.5 \pm 0.57
Albumin	ALB	g/dL	4.2 \pm 0.27	4.2 \pm 0.30	4.2 \pm 0.28
Globulin	GLB	g/dL	3.0 \pm 0.36	3.5 \pm 0.50	3.3 \pm 0.50
Albumin/Globulin Ratio	A/G ratio	-	1.4 \pm 0.18	1.2 \pm 0.17	1.3 \pm 0.20
Glucose	GLU	mg/dL	76.2 \pm 18.41	71.1 \pm 13.65	73.6 \pm 16.32
Alanine Aminotransferase	ALT	IU/L	59.3 \pm 33.95	61.7 \pm 41.01	60.5 \pm 37.53
Aspartate Aminotransferase	AST	IU/L	48.5 \pm 17.32	47.9 \pm 47.57	48.2 \pm 35.76
Total Bilirubin	T-BIL	mg/dL	0.2 \pm 0.07	0.2 \pm 0.07	0.2 \pm 0.07
Urea Nitrogen	BUN	mg/dL	24.6 \pm 4.55	21.2 \pm 4.47	23.2 \pm 4.73
Creatinine	CREA	mg/dL	0.6 \pm 0.13	0.9 \pm 0.19	0.8 \pm 0.23
Cholesterol	CHOL	mg/dL	129.5 \pm 31.12	107.4 \pm 23.05	118.4 \pm 29.39
Triglycerides	TG	mg/dL	40.3 \pm 27.20	26.7 \pm 15.13	33.4 \pm 22.92
Calcium	Ca	mg/dL	10.0 \pm 0.46	9.6 \pm 0.57	9.8 \pm 0.54
Phosphorus	P	mg/dL	5.2 \pm 0.97	5.3 \pm 1.05	5.3 \pm 1.01
Sodium	Na	mEq/L	150.4 \pm 2.60	150.3 \pm 2.14	150.3 \pm 2.37
Potassium	K	mEq/L	5.0 \pm 0.65	5.3 \pm 0.88	5.2 \pm 0.78
Chloride	Cl	mEq/L	108.2 \pm 2.61	107.6 \pm 2.43	107.9 \pm 2.53

showed that white blood cell (WBC) and mean corpuscular hemoglobin (MCH) values were lower than that of others whereas red blood cell (RBC) was higher. Basophil and platelet were highest in China monkey references. In Cambodia origin, neutrophil, monocyte, eosinophil, and mean corpuscular hemoglobin concentration (MCHC) showed relatively higher than other references and lymphocyte, hematocrit, and mean corpuscular volume (MCV) were low.

Biochemistry values were also compared between younger (19-28 months old) and older (39-72 months old) Cynomolgus monkeys (Table 4). Both groups had similar values in majority of serum biochemistry. However, the younger group showed higher triglycerides than the older group while alanine aminotransferase (ALT) was higher in the older group. Moreover, the values of glucose, ALT, AST, cholesterol, triglycerides showed big differences among individuals and the older group

Table 5. Serum biochemistry values comparison with other references

Items	Units	KFDA	Mauritius	China	Cambodia
TP	g/dL	6.8-9.2	7.9±0.28	7.57±0.49	7.5±0.57
ALB	g/dL	-	4.89±0.287	4.57±0.26	4.2±0.28
GLB	g/dL	-	3.0±0.20	-	3.3±0.50
A/G ratio	-	0.9-1.7	-	1.53±0.12	1.3±0.20
GLU	mg/dL	60-137	73±9.8	78.51±12.51	73.6±16.32
ALT	IU/L	10-83	38±12.4	45.39±12.94	60.5±37.53
AST	IU/L	7-34	43±4.4	50.18±11.09	48.2±35.76
T-BIL	mg/dL	-	0.39±0.079	0.24±0.04	0.2±0.07
BUN	mg/dL	16.2-33.8	48±8.3	17.78±3.26	23.2±4.73
CREA	mg/dL	0.46-0.99	0.85±0.088	0.82±0.08	0.8±0.23
CHOL	mg/dL	79-193	-	-	118.4±29.39
TG	mg/dL	18-68	65±14.9	22.07±6.22	33.4±22.92
Ca	mg/dL	9.0-11.6	11.6±0.43	9.34±0.34	9.8±0.54
P	mg/dL	3.4-6.5	-	-	5.3±1.01
Na	mEq/L	145-161	157±3.24	150.46±1.99	150.3±2.37
K	mEq/L	4.1-6.2	6.28±0.666	5.03±0.35	5.2±0.78
Cl	mEq/L	104-117	-	108.0±1.42	107.9±2.53

had relatively more variations. Comparing serum biochemistry value with other references is represented in Table 5. The urea nitrogen and triglycerides showed highest value in Mauritius origin and AST showed high level in all references, especially in Chinese origin. Creatinine value of the Cynomolgus monkeys of Cambodian origin was lower than that of other references. Sodium and Potassium values of Mauritius monkeys were relatively high.

Determination of Blood Type in Cynomolgus Monkeys

Healthy human blood was used for determining the blood type of the Cynomolgus monkey. Human RBC was mixed with serum of Cynomolgus monkey and agglutination was monitored (Figure 2). The agglutination was only detected in the mixture of Human RBC of blood type A, the Cynomolgus monkey had anti-A which means it was blood type B. Likewise, the agglutination in Human RBC of blood type B represented blood type A and both agglutination of Human RBC blood type A and B had blood type O. Lastly, blood type AB was determined when the agglutination was not observed. Moreover, the randomly selected monkeys and minor agglutination results were additionally confirmed by using DNA analysis and Immunohistochemistry method as previously described [19]. The blood type percentage in Cynomolgus monkey resulted approximately 15.6 (A), 33.3 (B), 44.2 (AB) and 6.9% (O), respectively. It could be concluded that main blood types of Cynomolgus monkeys in the

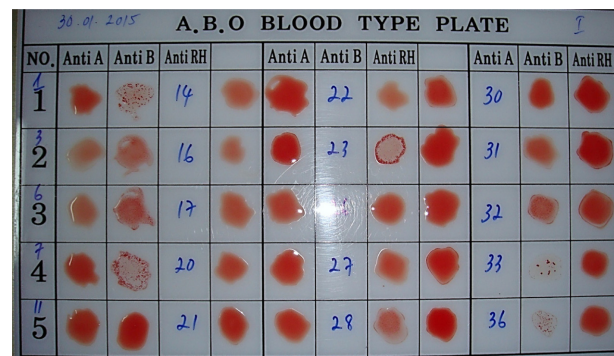


Figure 2. Determination of blood type in Cynomolgus monkeys. Human RBC was mixed with serum of Cynomolgus monkey and observed the agglutination.

breeding facility operated by Orient Cam in Cambodia were type AB and B.

Discussion

The Cynomolgus monkey has been used in various biomedical fields as a valuable animal model for aging disease, cardiovascular disease, Alzheimer's disease, bone loss, osteoarthritis, obesity, and diabetes due to its human homology [2]. Animal reference values have a vital role in evaluating non-clinical findings and selecting healthy subjects in biomedical research. These monkeys originating from different areas of Southeast Asia, are now purpose-bred in a limited number of breeding centers in different geographical locations [6]. Among various geographical locations, Cynomolgus monkeys

of Cambodia origin have been introduced to Korean researchers since 2013. Biological background data of Cynomolgus monkeys have been provided from various origins (Mauritius [5], China [20], etc.), however, the biological reference values of Cambodia origin Cynomolgus monkeys have not been provided yet. Accordingly, the main purpose of this study was providing biological reference values of Cambodia origin Cynomolgus monkey to biomedical researchers. Therefore, in this study, we provided information of the average body weight of 2,518 Cynomolgus monkeys and the hematology and serum biochemistry of 119 male Cynomolgus monkeys of Cambodia origin. Additionally, the blood types of 642 Cynomolgus monkeys were determined from Orient Cam in Cambodia.

Regarding body weight of animals aging from 24 to 36 months, 2.56 ± 0.345 kg resulted as the average for the male group and 2.43 ± 0.330 kg for the female group. Both genders had similar average body weight which demonstrated that these groups were ideal subjects to be experimented for efficacy and toxicity evaluation. In transplantation study, especially the allo- or xeno-transplantation, animal research models with relatively high body weight (5 kg) have been commonly used [21, 22]. This is why researchers have the tendency to prefer male primates over female. Male monkeys between the ages of 60 to 84 months with average body weight being approximately 5 kg would be the appropriate research models for transplantations. It was reported that the body weight could be different according to origin-related growing conditions in the breeding centers such as the available exercise area. For example, the monkeys originating from Mauritius and Philippines, although their ages were similar, showed some variations [6]. Therefore, body weight data assorted according to sex, age, and geographical origin is essential to properly conduct biomedical researches.

This study also performed evaluation of hematology and biochemistry values by comparing between younger and older male Cynomolgus monkeys. Many studies demonstrated that difference in sex had no effect on hematology though RBC analytes (RBC count, HGB, HCT) were usually reported to be lower in females [5,15,23,24]. Therefore, we conducted hematology and biochemistry study only using male monkeys according to age. In this study, age had a slight effect neutrophils, lymphocytes, Hct and MCV. It was reported that the RBC count decreased with age whereas Hb, MCV and

MCH tended to increase in Vietnamese monkeys [25]. Our results, however, showed no signs of RBC decrease between younger and older male Cynomolgus monkeys, only a slight increase in MCV. The increased MCV result is consistent with previous report which shows higher MCV level in sexual mature male animals [6]. Moreover, a slight increase in Hct was observed in older age monkeys of Cambodia origin as it did in sexually matured Cynomolgus males [24]. When the derived data was compared to other references, there were some differentiations also observed. The hematological values of neutrophil, monocyte, eosinophil, and MCHC of Cambodia origin monkeys were higher than those of other references whereas the values of lymphocyte, basophil, hemoglobin, hematocrit, and MCV of Cambodian monkeys were lower. Interestingly, RBC counts were higher in Mauritius monkeys than Cambodia origin. It is consistent with previous reports which shows Cynomolgus monkeys from Mauritius have higher RBC counts than those of Southeast Asia [6,26].

The values of glucose, ALT, blood urea nitrogen, and creatinine varied among animals. Because urea levels could be changed by protein content of the intake food, the variations occurred in accordance with different conditions of food consumption and environment. The older male groups showed higher creatinine values than younger male group and the average creatinine value of Cambodia origin was lower than other origins. Because it has been reported that creatinine induced various models of hepatotoxicity, the lower value is important [15]. The difference of hematology and serum biochemistry values observed in monkeys from different country of origin might have been caused by their exposure to different dietary constituents and environment. Moreover, many factors including climate, genetic background and age of the animals could be affect the results. Because background data of hematology and biochemistry values reported from different researchers were obtained under various environmental and experimental conditions throughout the data collection, we emphasize that the reference values of Cynomolgus monkeys of Cambodia origin are important information to biomedical researchers who will use those as an animal model.

It is imperative to match up blood types of the monkeys in allo- and xeno-transplantation. Like human beings, Cynomolgus monkeys also have their own blood types. However, it is impossible to define blood types with human anti-A and anti-B sold in the market,

because red blood cell of *Cynomolgus* monkey has no epitope which is able to be recognized by human anti-A and anti-B. Therefore, alternative method was necessary and we adopted indirect method in determining the blood type by monitoring reactivity of agglutination after serum of *Cynomolgus* monkey was mixed with human RBC. However, this antisera of this test has a drawback from age-associated differences in the levels of serum antibody secreted in each animal [27]. Therefore, it is necessary to other validation support such as DNA analysis and immunohistochemistry (IHC) for determining the blood type of *Cynomolgus* monkeys [19]. Although the hamagglutination assay has some limitations, this method is very simple and quick (few seconds), cost-effective, reliable on many occasions, and is noted for high applicability in the field, especially when large number of animals are to be tested. In this study, the procedure of determining their blood type was in compliance with the SOP which was established in Genia, "Procedure of Blood Type Determination" followed by Orient Cam. The result showed that approximately 44.2% of *Cynomolgus* monkeys were blood type AB and 33.3% of them were blood type B. The percentage of polymorphic in ABO blood types was B>AB>A>O, respectively, which showed similarities to those of *Cynomolgus* monkeys from Philippines [28]. We also confirmed the blood type on randomly selected monkeys and in case of minor agglutination samples were performed to reinspect for identification of blood type by using DNA analysis method and IHC [19]. Most results were well consistent with hemagglutination assay even though some animals showed different results. In the case of organ transplantation studies, researchers used same blood type of animal models with no specific blood type preferred in their study. Therefore, when perform transplantation study, it is necessary to cross-check their blood types. Orient Cam has a large pool of blood type B and AB monkeys which may provide researchers with advantages for screening monkeys that best suit their research demands.

In conclusion, this study provides basic biological reference data for researchers who would willingly use *Cynomolgus* monkeys of Cambodia origin in their studies. Moreover, as *Cynomolgus* monkeys of Cambodia origin are newly introduced in Korea, this study could help the researchers by providing basic background data of this origin.

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Conflict of interests The authors declare that there is no financial conflict of interests to publish these results.

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