BIO Integration

Establishment of Hematological and Serum Biochemical Parameters in 4,834 Healthy Cynomolgus Monkeys (*Macaca fascicularis*)

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Abstract

Background: In China and other countries, cynomolgus monkeys are commonly used experimental animal models in biomedical research. Reference hematologic and biochemical parameters must be established to evaluate healthy cynomolgus monkeys and investigate drug safety in non-clinical research. In the present study, data from 4,834 healthy cynomolgus monkeys were used to establish a reference for various age groups.

Methods: A total of 4834 healthy cynomolgus monkeys (649 males and 4185 females) were selected and divided into six groups according to age and sex. Twenty hematological parameters and 17 serum biochemical parameters were detected, and the effects of age and sex were analyzed.

Results: A reference database of hematological and biochemical parameters was established according to age (six groups) and sex (male and female). Significant differences were observed in White blood cell, Red blood cell, Hemoglobin, Hematocrit, Total protein, Albumin, Total bilirubin, Direct bilirubin, Alkaline phosphatase, Glucose, Blood urea nitrogen, Creatinine, Calcium, Total cholesterol, Triglyceride, and Lactate dehydrogenase values according to age in the juvenile and young groups ($P<0.05$). Significant differences between males and females were observed in Red blood cell, Hemoglobin, Hematocrit, Mean corpuscular hemoglobin concentration, White blood cell, Lymphocyte, Lymphocyte percentage, Monocyte, Monocyte percentage, Basophil, and Basophil percentage in most age groups ($P<0.05$).

Conclusion: Reference ranges for healthy cynomolgus monkeys were established in different age and sex groups in this study. The findings may be useful in clinical care and non-human-primate research.

Keywords

Biochemistry, cynomolgus monkeys, hematology, *Macaca fascicularis*.

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Introduction

Abundant non-human-primate resources exist in China. Cynomolgus monkeys (*Macaca fascicularis*) are highly genetically similar to humans, thus making them important experimental animal models in biomedical research fields [1], including cerebral diseases, traditional Chinese medicine, molecular genetics, pharmacokinetics, and toxicology [2–6]. Given the important roles of these monkeys in research, reference hematologic and biochemical parameters must be established to aid in the assessment of animal health status, and investigation of the effects induced by drugs or treatments.

A prior study has reported the hematological and biochemical parameters of cynomolgus monkeys according to age and sex [7]. Reference clinical pathology data have been reported in another study [8]. A previous report has also investigated the reference values of hematological and biochemical parameters, and compared the differences between two species (cynomolgus monkeys and Rhesus monkeys) [9]. However, although many studies have reported hematologic and biochemical parameters of non-human primates [7, 9–14], only several studies have provided reference data from an extensive number of animals, ranging in age from juvenile to old.

Therefore, his study was aimed at establishing a reference database of hematologic and biochemical parameters of cynomolgus monkeys according to age and sex, to provide a basis for selecting healthy

animals for constructing disease models, and for improving the effectiveness of disease diagnosis and treatment.

Materials and methods

Animals

A total of 4,834 healthy cynomolgus monkeys (649 males and 4,185 females) 1–22 years of age (Y), were obtained from three monkey farms at Guangdong Blooming-Spring Biological Technology Development Co., Ltd.; the Kunming Institute of Zoology; and the Huazheng Laboratory Animal Breeding Centre. We divided the animals into six age groups: animals 1–3 Y were in the juvenile group, those 4–6 Y were in the young group, those 7–9 Y were in the adult group, those 10–12 Y were in the middle-age group, those 13–15 Y were in the old group, and those older than 15 Y were in the very old group. All cynomolgus monkeys collected from the three monkey farms were of Chinese origin and were determined to be healthy in annual examinations including their hair, behavior, spirit, activity, skin, joints, excretion, blood parameters, and microbial parameters. All animals had tested negative for tuberculosis and Herpes B virus. In addition, all female monkeys were non-pregnant. The present study and all procedures were approved by the animal care and use committee of Kunming Institute of Zoology, Chinese Academy of Sciences (approval No: IACUC 16006). In addition, all experiments in monkeys from the three monkey farms were conducted in accordance with the Guide for the Care and Use of Laboratory Animals [15]. Hematological and biochemical data were gathered during routine annual quarantine and health monitoring from 2015 to 2017. No animals were sacrificed in this study.

Blood sample collection and preparation

All monkeys were fasted overnight for at least 8 hours before blood was drawn. The males were anesthetized with ketamine hydrochloride, and the females were conscious; 3-mL blood samples were drawn from cephalic veins with syringes. First, the 1-mL blood sample was transferred into a tube containing ethylenediamine tetraacetic acid dipotassium tube, and the blood was adequately mixed by inversion of the tube at least five times. Then 2-mL of the remaining blood sample was transferred into a vacuum drying tube, and kept at room temperature for 30 min. The serum was separated by centrifugation at 1600 g for 15 min.

Hematological analysis

Hematological analysis was performed with a Sysmex XT-2000iv automated hematology analyzer. The following parameters were analyzed: white blood cell count (WBC, 10^9/L), red blood cell count (RBC, 10^{12}/L), hemoglobin (HGB, g/L), hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin mass (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/L), platelets (PLT, 10^9/L), mean platelet volume (MPV, fL), plateletcrit (PCT, %), neutrophils (NEUT, 10^9/L), lymphocytes (LYMPH, 10^9/L), monocytes (MONO, 10^9/L), eosinophils (EO, 10^9/L), basophils (BASO, 10^9/L), neutrophil percentage (NEUT%, %), lymphocyte percentage (LYMPH%, %), monocyte percentage (MONO%, %), eosinophil percentage (EO%, %), and basophil percentage (BASO%, %).

Biochemical analysis

Biochemical analysis was performed with a HITACHI7020 automated biochemical analyzer. The following parameters were analyzed: alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), total protein (TP, g/L), albumin (ALB, g/L), total bilirubin (TBIL, μmol/L), direct bilirubin (DBIL, μmol/L), alkaline phosphatase (ALP, U/L), blood urea nitrogen (BUN, mmol/L), creatinine (CREA, μmol/L), glucose (GLU, mmol/L), total cholesterol (CHOL, mmol/L), triglyceride (TG, mmol/L), high-density-lipoprotein cholesterol (HDL-C, mmol/L), low-density-lipoprotein cholesterol (LDL-C, mmol/L), lactate dehydrogenase (LDH, U/L), calcium (Ca, mmol/L), and phosphorus (P, mmol/L).

Statistical analysis

The hematological and biochemical results were statistically analyzed in SPSS version 22.0, with two-tailed Student's t-tests and Kruskal-Wallis tests to detect significant differences between age groups for each sex, and between sexes in each age group. The data are reported as mean ± SD, and a p-value less than 0.05 was considered statistically significant.

Results

Hematological and biochemical values

The values of hematological parameters are presented in **Table 1**. In the juvenile and young groups, age had a statistically significant effect on RBC, HGB, HCT, MCV, MCH, NEUT%, LYMPH%, and BASO% ($P<0.05$). Compared with the adult and old groups, the WBC, RBC, HGB, and HCT values were significantly higher in the juvenile and young age groups ($P<0.05$). Significant differences between males and females were observed for RBC, HGB, HCT, MCHC, WBC, LYMPH, LYMPH%, MONO, MONO%, BASO, and BASO% in most age groups ($P<0.05$).

The biochemical values are presented in **Table 2**. Significant differences were observed in TP, ALB, TBIL, DBIL, ALP, GLU, BUN, CREA, Ca, CHOL, TG, and LDH according to age in the juvenile and young groups

Table 1 Hematological-Parameter Values by Sex and Age

Parameters (units)	Age 1–3 Y			Age 4–6 Y		
	Female (n=99)	Male (n=32)	Both (n=131)	Female (n=729)	Male (n=56)	Both (n=785)
WBC (10^9/L)	17.05 ± 3.99	18.27 ± 3.41	17.38 ± 3.86	15.79 ± 4.28	14.93 ± 4.56a	15.73 ± 4.30
RBC (10^{12}/L)	6.03 ± 0.65	6.53 ± 0.38*	6.14 ± 0.63	5.58 ± 0.52a	5.88 ± 0.62*a	5.60 ± 0.53
HGB (g/L)	141.0 ± 12.0	153.4 ± 6.7*	144.0 ± 12.2	132.8 ± 10.8a	143.1 ± 12.4*a	133.5 ± 11.2
HCT (%)	46.43 ± 3.93	49.70 ± 1.75*	47.21 ± 3.79	44.15 ± 3.57a	47.18 ± 4.06*acd	44.35 ± 3.68
MCV (fL)	77.28 ± 4.59	76.04 ± 3.76	76.99 ± 4.43	79.07 ± 3.75a	79.97 ± 3.83a	79.13 ± 3.76
MCH (pg)	23.47 ± 1.38	23.50 ± 1.15	23.48 ± 1.32	23.88 ± 1.17a	24.50 ± 1.39*a	23.93 ± 1.20
MCHC (g/L)	304.2 ± 9.9	308.7 ± 6.8*	305.3 ± 9.5	301.4 ± 9.8	304.9 ± 11.1*	301.7 ± 10.0
PLT (10^9/L)	416.5 ± 84.8	409.7 ± 80.4	414.9 ± 83.5	375.1 ± 90.4a	373.2 ± 90.0	374.9 ± 90.3
MPV (fL)	11.58 ± 1.00	11.75 ± 1.38	11.62 ± 1.09	11.73 ± 1.11	11.30 ± 1.27*	11.70 ± 1.12
PCT (%)	0.49 ± 0.09	0.48 ± 0.06	0.48 ± 0.08	0.44 ± 0.10a	0.42 ± 0.09	0.44 ± 0.10
NEUT (10^9/L)	7.34 ± 3.40	5.62 ± 2.15*	6.89 ± 3.20	8.49 ± 3.83	7.57 ± 3.40	8.42 ± 3.81
LYMPH (10^9/L)	7.20 ± 2.14	8.66 ± 1.55*	7.38 ± 2.12	5.48 ± 2.39a	5.65 ± 2.41a	5.49 ± 2.39
MONO (10^9/L)	1.01 ± 0.36	1.05 ± 0.26	1.02 ± 0.34	0.84 ± 0.34a	0.86 ± 0.34	0.84 ± 0.34
EO (10^9/L)	0.22 ± 0.15	0.24 ± 0.17	0.22 ± 0.15	0.14 ± 0.14a	0.16 ± 0.14	0.14 ± 0.14
BASO (10^9/L)	0.01 ± 0.01	0.02 ± 0.01*	0.02 ± 0.01	0.01 ± 0.01a	0.01 ± 0.01a	0.01 ± 0.01
NEUT% (%)	45.08 ± 17.08	31.12 ± 6.87*	41.96 ± 16.45	56.76 ± 18.04a	51.95 ± 18.33a	56.41 ± 18.09
LYMPH% (%)	47.36 ± 16.09	60.78 ± 5.87*	50.30 ± 15.50	36.23 ± 16.41a	41.54 ± 17.37*a	36.61 ± 16.53
MONO% (%)	5.84 ± 1.91	6.22 ± 1.78	5.93 ± 1.88	5.46 ± 2.21	5.40 ± 1.82	5.45 ± 2.18
EO% (%)	1.24 ± 0.93	1.30 ± 1.00	1.25 ± 0.94	0.91 ± 0.90	1.05 ± 0.88	0.92 ± 0.90
BASO% (%)	0.09 ± 0.06	0.11 ± 0.04	0.10 ± 0.06	0.07 ± 0.05a	0.07 ± 0.05a	0.07 ± 0.05

*, Significant (P<0.05) difference with respect to sex.
a, Significant (P<0.05) difference with respect to age group 1–3 Y.
b, Significant (P<0.05) difference with respect to age group 4–6 Y.
c, Significant (P<0.05) difference with respect to age group 7–9 Y.
d, Significant (P<0.05) difference with respect to age group 10–12 Y.
e, Significant (P<0.05) difference with respect to age group 13–15 Y.

Table 1 Continued

Parameters (units)	Age 7–9 Y			Age 10–12 Y		
	Female (n=661)	Male (n=82)	Both (n=743)	Female (n=1356)	Male (n=198)	Both (n=1563)
WBC (10^9/L)	14.04 ± 4.15ab	12.95 ± 4.02*a	13.92 ± 4.15	13.00 ± 3.82abc	11.35 ± 3.54*abc	12.79 ± 3.82
RBC (10^{12}/L)	5.59 ± 0.53a	5.89 ± 0.47*a	5.62 ± 0.53	5.59 ± 0.53a	5.73 ± 0.49*a	5.61 ± 0.53
HGB (g/L)	133.3 ± 11.22a	143.2 ± 9.5*a	134.4 ± 11.5	132.1 ± 11.1a	138.2 ± 9.9*abc	132.9 ± 11.1
HCT (%)	44.05 ± 3.64a	46.73 ± 3.31*a	44.35 ± 3.70	43.96 ± 3.52a	45.34 ± 3.10*abc	44.14 ± 3.50
MCV (fL)	78.82 ± 3.74a	78.97 ± 3.66a	78.84 ± 3.73	78.75 ± 3.93a	79.29 ± 3.87a	78.82 ± 3.93
MCH (pg)	23.93 ± 1.18a	24.44 ± 1.17*a	23.98 ± 1.19	23.69 ± 1.25bc	24.17 ± 1.24*	23.75 ± 1.26
MCHC (g/L)	303.1 ± 9.2b	308.0 ± 7.2*	303.7 ± 9.1	300.1 ± 9.1abc	304.3 ± 8.4*c	300.6 ± 9.1
PLT (10^9/L)	366.2 ± 91.5a	369.3 ± 75.7	366.5 ± 89.9	332.9 ± 90.7abc	313.4 ± 80.2*abc	330.5 ± 89.6
MPV (fL)	11.73 ± 1.15	11.24 ± 1.17*	11.68 ± 1.16	11.87 ± 1.12	11.43 ± 1.10*	11.81 ± 1.13
PCT (%)	0.43 ± 0.09a	0.42 ± 0.08a	0.43 ± 0.09	0.39 ± 0.09abc	0.36 ± 0.08*abc	0.39 ± 0.09
NEUT (10^9/L)	7.74 ± 3.64b	7.25 ± 3.33	7.69 ± 3.61	6.84 ± 3.60bc	6.34 ± 3.31	6.78 ± 3.57
LYMPH (10^9/L)	4.84 ± 2.09ab	4.28 ± 2.23*ab	4.77 ± 2.11	4.77 ± 2.10ab	3.88 ± 1.69*ab	4.66 ± 2.07
MONO (10^9/L)	0.80 ± 0.34a	0.70 ± 0.34*ab	0.79 ± 0.34	0.82 ± 0.34a	0.67 ± 0.29*ab	0.80 ± 0.34
EO (10^9/L)	0.14 ± 0.14a	0.14 ± 0.13a	0.14 ± 0.14	0.16 ± 0.15a	0.17 ± 0.15	0.16 ± 0.15
BASO (10^9/L)	0.01 ± 0.01ab	0.01 ± 0.01*ab	0.01 ± 0.01	0.01 ± 0.01ab	0.00 ± 0.00*ab	0.01 ± 0.01
NEUT% (%)	56.55 ± 17.21a	58.76 ± 18.76a	56.79 ± 17.39	53.08 ± 18.35abc	56.61 ± 17.30*a	53.53 ± 18.25
LYMPH% (%)	35.90 ± 15.32a	34.03 ± 17.52a	35.69 ± 15.57	38.32 ± 16.27ac	35.46 ± 15.19*a	37.95 ± 16.16
MONO% (%)	5.83 ± 2.19b	5.12 ± 2.09*	5.75 ± 2.19	6.27 ± 2.21bc	5.92 ± 2.14*c	6.22 ± 2.20
EO% (%)	1.05 ± 1.04	1.10 ± 1.01	1.06 ± 1.03	1.20 ± 1.15b	1.32 ± 1.10	1.21 ± 1.14
BASO% (%)	0.07 ± 0.05	0.05 ± 0.06*a	0.07 ± 0.05	0.08 ± 0.05b	0.05 ± 0.05*a	0.08 ± 0.05

*, Significant (P<0.05) difference with respect to sex.
a, Significant (P<0.05) difference with respect to age group 1–3 Y.
b, Significant (P<0.05) difference with respect to age group 4–6 Y.
c, Significant (P<0.05) difference with respect to age group 7–9 Y.
d, Significant (P<0.05) difference with respect to age group 10–12 Y.
e, Significant (P<0.05) difference with respect to age group 13–15 Y.

Table 1 Continued

Parameters (units)	Age 13–15 Y			Age above 15 Y		
	Female (n=837)	Male (n=215)	Both (n=1052)	Female (n=494)	Male (n=66)	Both (n=560)
WBC (10^9/L)	12.60 ± 3.80abc	11.64 ± 3.82*ab	12.40 ± 3.82	11.81 ± 3.41abcde	11.39 ± 3.30ab	11.76 ± 3.40
RBC (10^{12}/L)	5.66 ± 0.56abd	5.68 ± 0.50ac	5.67 ± 0.54	5.75 ± 0.59abcd	5.73 ± 0.45a	5.74 ± 0.58
HGB (g/L)	133.0 ± 11.8a	137.7 ± 11.0*ac	134.0 ± 11.8	134.8 ± 13.3abd	140.0 ± 10.8*a	135.5 ± 13.1
HCT (%)	44.33 ± 3.65a	45.08 ± 3.52*abc	44.49 ± 3.63	45.03 ± 3.99abcde	45.68 ± 3.24a	45.11 ± 3.91
MCV (fL)	78.47 ± 3.93b	79.52 ± 3.61*a	78.68 ± 3.89	78.40 ± 3.94b	79.45 ± 2.98*a	78.53 ± 3.85
MCH (pg)	23.56 ± 1.29bc	24.34 ± 1.18*a	23.72 ± 1.31	23.57 ± 1.34bc	24.53 ± 1.23*a	23.68 ± 1.36
MCHC (g/L)	299.4 ± 9.2abc	305.8 ± 8.7*	300.7 ± 9.4	300.0 ± 9.9ac	307.5 ± 7.7*	300.9 ± 10.0
PLT (10^9/L)	342.2 ± 93.8abc	336.9 ± 92.0ac	341.1 ± 93.4	338.5 ± 88.8abc	311.2 ± 68.2abc	335.4 ± 87.1
MPV (fL)	11.78 ± 1.18	11.22 ± 1.10*	11.66 ± 1.19	11.81 ± 1.14	11.34 ± 1.32*	11.76 ± 1.17
PCT (%)	0.40 ± 0.10abc	0.37 ± 0.09*abc	0.40 ± 0.09	0.40 ± 0.09abc	0.35 ± 0.06*abc	0.40 ± 0.09
NEUT (10^9/L)	6.38 ± 3.34bcd	6.38 ± 3.56	6.38 ± 3.39	5.83 ± 3.20abcd	6.14 ± 3.30	5.86 ± 3.21
LYMPH (10^9/L)	4.86 ± 2.11ab	4.14 ± 1.95*ab	4.71 ± 2.10	4.72 ± 1.99ab	4.28 ± 1.89ab	4.66 ± 1.98
MONO (10^9/L)	0.84 ± 0.35a	0.73 ± 0.31*a	0.82 ± 0.35	0.86 ± 0.34a	0.72 ± 0.33*a	0.84 ± 0.34
EO (10^9/L)	0.19 ± 0.17bcd	0.18 ± 0.15	0.19 ± 0.16	0.18 ± 0.16bcd	0.21 ± 0.17	0.19 ± 0.16
BASO (10^9/L)	0.01 ± 0.01ab	0.01 ± 0.00*ab	0.01 ± 0.01	0.01 ± 0.01ab	0.00 ± 0.00*ab	0.01 ± 0.01
NEUT% (%)	50.33 ± 17.61bcd	53.87 ± 18.71*a	51.06 ± 17.89	49.30 ± 18.06bcd	52.35 ± 17.56a	49.66 ± 18.01
LYMPH% (%)	40.02 ± 15.49abc	37.41 ± 16.56*a	39.48 ± 15.74	40.73 ± 16.03abc	38.46 ± 15.75a	40.46 ± 16.00
MONO% (%)	6.66 ± 2.32abcd	6.30 ± 2.18*c	6.58 ± 2.30	7.14 ± 2.26abcde	6.25 ± 2.41*c	7.03 ± 2.30
EO% (%)	1.51 ± 1.29bcd	1.47 ± 1.13	1.50 ± 1.26	1.56 ± 1.29bcd	1.78 ± 1.26bc	1.59 ± 1.28
BASO% (%)	0.08 ± 0.05bc	0.06 ± 0.06*a	0.08 ± 0.05	0.08 ± 0.05b	0.05 ± 0.05*a	0.08 ± 0.05

*, Significant (P<0.05) difference with respect to sex.
a, Significant (P<0.05) difference with respect to age group 1–3 Y.
b, Significant (P<0.05) difference with respect to age group 4–6 Y.
c, Significant (P<0.05) difference with respect to age group 7–9 Y.
d, Significant (P<0.05) difference with respect to age group 10–12 Y.
e, Significant (P<0.05) difference with respect to age group 13–15 Y.

Table 2 Biochemical-Parameter Values by Sex and Age

Parameters (units)	Age 1–3 Y			Age 4–6 Y		
	Female (n=99)	Male (n=32)	Both (n=131)	Female (n=729)	Male (n=56)	Both (n=785)
ALT (U/L)	28.07 ± 12.24	29.40 ± 7.51	28.42 ± 11.18	26.47 ± 17.52	26.66 ± 15.46	26.48 ± 17.37
AST (U/L)	51.28 ± 11.37	55.82 ± 12.80	52.44 ± 11.85	51.57 ± 14.62	47.16 ± 14.22*a	51.24 ± 14.62
TP (g/L)	70.38 ± 6.64	68.39 ± 3.92	69.83 ± 6.06	76.43 ± 7.17a	75.28 ± 6.49a	76.35 ± 7.13
ALB (g/L)	39.50 ± 5.16	40.55 ± 2.71	39.79 ± 4.63	41.32 ± 5.81a	43.29 ± 5.04*a	41.47 ± 5.78
TBIL (μmol/L)	4.07 ± 2.40	1.22 ± 0.58*	3.33 ± 2.43	5.22 ± 1.80a	3.93 ± 2.64*a	5.13 ± 1.90
D-BIL (μmol/L)	1.04 ± 0.48	0.76 ± 0.36*	0.97 ± 0.47	1.47 ± 0.68a	1.21 ± 0.54*a	1.45 ± 0.67
ALP (U/L)	348.1 ± 190.7	587.5 ± 123.1*	406.3 ± 204.1	199.5 ± 91.0a	251.9 ± 155.8*a	202.8 ± 97.1
GLU (mmol/L)	4.95 ± 1.85	5.97 ± 1.24*	5.21 ± 1.77	4.03 ± 1.76a	4.23 ± 1.83a	4.04 ± 1.76
BUN (mmol/L)	7.30 ± 1.79	9.44 ± 0.85*	7.83 ± 1.86	6.71 ± 1.46a	7.03 ± 1.36a	6.73 ± 1.45
CREA (μmol/L)	61.19 ± 14.37	46.57 ± 10.45*	57.60 ± 14.88	76.06 ± 14.52a	93.89 ± 14.29*a	77.14 ± 15.12
Ca (mmol/L)	2.44 ± 0.14	2.41 ± 0.10	2.43 ± 0.13	2.49 ± 0.14a	2.51 ± 0.12a	2.49 ± 0.14
P (mmol/L)	1.67 ± 0.29	1.84 ± 0.24*	1.71 ± 0.29	1.59 ± 0.34	1.52 ± 0.32a	1.58 ± 0.34
CHOL (mmol/L)	2.44 ± 0.94	3.08 ± 0.63*	2.61 ± 0.91	2.81 ± 1.03a	2.61 ± 0.63a	2.79 ± 1.01
TG (mmol/L)	0.72 ± 0.37	1.01 ± 0.38*	0.80 ± 0.39	0.57 ± 0.25a	0.63 ± 0.40a	0.58 ± 0.26
HDL-C (mmol/L)	1.24 ± 0.47	1.52 ± 0.35*	1.31 ± 0.45	1.41 ± 0.53	1.40 ± 0.30	1.41 ± 0.52
LDL-C (mmol/L)	1.32 ± 0.53	1.60 ± 0.35*	1.39 ± 0.50	1.29 ± 0.55	1.17 ± 0.39a	1.28 ± 0.54
LDH (U/L)	536.1 ± 149.5	620.2 ± 138.6*	556.7 ± 150.7	471.0 ± 207.7a	475.9 ± 221.7a	471.3 ± 208.6

*, Significant (P<0.05) difference with respect to sex.
a, Significant (P<0.05) difference with respect to age group 1–3 Y.
b, Significant (P<0.05) difference with respect to age group 4–6 Y.
c, Significant (P<0.05) difference with respect to age group 7–9 Y.
d, Significant (P<0.05) difference with respect to age group 10–12 Y.
e, Significant (P<0.05) difference with respect to age group 13–15 Y.

Table 2 Continued

Parameters (units)	Age 7–9 Y			Age 10–12 Y		
	Female (n=661)	Male (n=82)	Both (n=743)	Female (n=1365)	Male (n=198)	Both (n=1563)
ALT (U/L)	27.22 ± 18.21	26.70 ± 17.65	27.16 ± 18.14	22.40 ± 15.31abc	26.66 ± 15.46	22.03 ± 15.12
AST (U/L)	46.97 ± 13.26ab	48.49 ± 13.39	47.13 ± 13.27	42.40 ± 12.61abc	47.16 ± 14.22*a	42.11 ± 12.45
TP (g/L)	77.14 ± 6.73a	78.74 ± 5.17*ab	77.31 ± 6.60	78.30 ± 6.54abc	75.28 ± 6.49a	78.35 ± 6.38
ALB (g/L)	41.92 ± 5.38a	46.13 ± 3.53*ab	42.39 ± 5.37	40.98 ± 5.02c	43.29 ± 5.04*a	41.41 ± 5.04
TBIL (μmol/L)	5.19 ± 1.71a	4.99 ± 1.89ab	5.17 ± 1.73	4.92 ± 1.80abc	3.93 ± 2.64*a	4.88 ± 1.78
D-BIL (μmol/L)	1.43 ± 0.62a	1.48 ± 0.67a	1.44 ± 0.62	1.62 ± 0.72abc	1.21 ± 0.54*a	1.61 ± 0.71
ALP (U/L)	154.3 ± 58.5ab	138.3 ± 50.6*ab	152.5 ± 57.9	148.9 ± 54.9ab	251.9 ± 155.8*a	144.3 ± 54.6
GLU (mmol/L)	4.16 ± 1.70a	3.54 ± 1.25*ab	4.09 ± 1.67	4.14 ± 1.57a	4.23 ± 1.83a	4.07 ± 1.54
BUN (mmol/L)	6.44 ± 1.50ab	6.98 ± 1.35*a	6.50 ± 1.50	6.42 ± 1.50ab	7.03 ± 1.36a	6.48 ± 1.47
CREA (μmol/L)	77.15 ± 14.23a	104.1 ± 13.2*ab	79.36 ± 15.96	74.46 ± 14.86ac	93.89 ± 14.29*a	77.02 ± 16.69
Ca (mmol/L)	2.50 ± 0.15a	2.55 ± 0.11*a	2.50 ± 0.15	2.47 ± 0.14c	2.51 ± 0.12a	2.47 ± 0.14
P (mmol/L)	1.46 ± 0.32ab	1.49 ± 0.26a	1.47 ± 0.31	1.54 ± 0.33abc	1.52 ± 0.32a	1.53 ± 0.33
CHOL (mmol/L)	3.01 ± 0.99ab	2.98 ± 0.65b	3.01 ± 0.96	3.08 ± 1.01ab	2.61 ± 0.63a	3.06 ± 0.98
TG (mmol/L)	0.71 ± 0.36b	0.70 ± 0.53a	0.71 ± 0.38	0.79 ± 0.37bc	0.63 ± 0.40a	0.78 ± 0.38
HDL-C (mmol/L)	1.54 ± 0.51ab	1.60 ± 0.37b	1.55 ± 0.50	1.54 ± 0.52ab	1.40 ± 0.30	1.54 ± 0.50
LDL-C (mmol/L)	1.33 ± 0.56	1.27 ± 0.48a	1.33 ± 0.55	1.40 ± 0.55b	1.17 ± 0.39a	1.38 ± 0.54
LDH (U/L)	467.7 ± 216.9a	416.8 ± 205.0a	462.2 ± 216.1	378.4 ± 161.1abc	475.9 ± 221.7a	377.3 ± 163.3

*, Significant (P<0.05) difference with respect to sex.
a, Significant (P<0.05) difference with respect to age group 1–3 Y.
b, Significant (P<0.05) difference with respect to age group 4–6 Y.
c, Significant (P<0.05) difference with respect to age group 7–9 Y.
d, Significant (P<0.05) difference with respect to age group 10–12 Y.
e, Significant (P<0.05) difference with respect to age group 13–15 Y.

Table 2 Continued

Parameters (units)	Age 13–15 Y			Age above 15 Y		
	Female (n=837)	Male (n=215)	Both (n=1052)	Female (n=494)	Male (n=66)	Both (n=560)
ALT (U/L)	24.81 ± 16.79d	21.15 ± 14.73*a	24.06 ± 16.44	24.10 ± 16.30	25.50 ± 16.10	24.26 ± 16.27
AST (U/L)	42.31 ± 11.89abc	41.63 ± 11.70abc	42.17 ± 11.85	42.18 ± 11.53abc	45.26 ± 13.42*ad	42.55 ± 11.80
TP (g/L)	78.40 ± 6.43abc	77.56 ± 5.37*a	78.23 ± 6.23	78.77 ± 6.52abc	77.72 ± 5.01a	78.64 ± 6.36
ALB (g/L)	41.14 ± 4.82	42.57 ± 4.12*cd	41.44 ± 4.71	40.73 ± 4.76c	43.07 ± 3.38*c	41.02 ± 4.67
TBIL (μmol/L)	4.82 ± 1.69abc	4.48 ± 1.53*a	4.75 ± 1.66	4.71 ± 1.68abc	4.80 ± 1.68a	4.72 ± 1.68
D-BIL (μmol/L)	1.45 ± 0.66ad	1.48 ± 0.67a	1.46 ± 0.67	1.36 ± 0.67ad	1.31 ± 0.65ad	1.35 ± 0.67
ALP (U/L)	148.5 ± 54.1ab	120.0 ± 44.1*ab	142.6 ± 53.4	148.1 ± 56.7ab	108.7 ± 36.9*ab	143.2 ± 56.1
GLU (mmol/L)	4.48 ± 1.60bcd	3.85 ± 1.38*a	4.35 ± 1.57	4.80 ± 1.67bcde	4.38 ± 1.52acd	4.75 ± 1.66
BUN (mmol/L)	6.56 ± 1.53a	6.72 ± 1.39a	6.59 ± 1.51	6.69 ± 1.58ad	6.83 ± 1.32a	6.71 ± 1.55
CREA (μmol/L)	72.06 ± 15.14abcd	97.60 ± 16.72*ac	76.57 ± 18.24	69.49 ± 15.86abcde	94.07 ± 14.73*acd	72.20 ± 17.52
Ca (mmol/L)	2.49 ± 0.14	2.46 ± 0.14*c	2.48 ± 0.14	2.49 ± 0.16	2.48 ± 0.11c	2.49 ± 0.15
P (mmol/L)	1.58 ± 0.32cd	1.41 ± 0.32*a	1.55 ± 0.32	1.62 ± 0.32cd	1.37 ± 0.30*a	1.59 ± 0.33
CHOL (mmol/L)	3.16 ± 0.97ab	2.72 ± 0.76*	3.07 ± 0.95	3.16 ± 0.97ab	2.71 ± 0.76*	3.10 ± 0.96
TG (mmol/L)	0.88 ± 0.46abcd	0.81 ± 0.47*	0.87 ± 0.46	0.91 ± 0.47abcd	0.94 ± 0.56bc	0.91 ± 0.48
HDL-C (mmol/L)	1.57 ± 0.50ab	1.43 ± 0.36*c	1.54 ± 0.48	1.57 ± 0.51ab	1.43 ± 0.39*	1.56 ± 0.50
LDL-C (mmol/L)	1.42 ± 0.53b	1.14 ± 0.47*a	1.36 ± 0.53	1.43 ± 0.52b	1.11 ± 0.44*a	1.39 ± 0.52
LDH (U/L)	410.0 ± 183.6abcd	389.5 ± 189.1*ab	405.8 ± 184.8	411.4 ± 190.8abcd	451.8 ± 229.4abd	416.0 ± 195.7

*, Significant (P<0.05) difference with respect to sex.
a, Significant (P<0.05) difference with respect to age group 1–3 Y.
b, Significant (P<0.05) difference with respect to age group 4–6 Y.
c, Significant (P<0.05) difference with respect to age group 7–9 Y.
d, Significant (P<0.05) difference with respect to age group 10–12 Y.
e, Significant (P<0.05) difference with respect to age group 13–15 Y.

(P<0.05). ALP values were significantly higher in the juvenile age group (1–3 Y) than the adult and old groups. TP values increased with age in the young age groups (1–6 Y) and became stable after adult age. Significant differences between males and females were observed for ALP and CREA in most age groups (P<0.05).

Effects of geographical location, breeding conditions and climatic factors on hematological and biochemical values in three monkey farms

All samples originated from three monkey farms: Guangdong Blooming-Spring Biological Technology Development Co., Ltd. (farm 1), the Kunming Institute of Zoology (farm 2), and the Huazheng Laboratory Animal Breeding Centre (farm 3). Because of the variations in the animals' age distribution among the three farms, we present a comparison of hematological and biochemical values in two age groups (4–6 Y and 7–9 Y) among the three farms (**Table 3** and **Table 4**). A significant difference in RBC, HGB, and HCT values was observed among the three farms in the 4–6 Y group (P<0.05; **Table 3**). In the 7–9 Y group, no significant differences were

Table 3 Comparison of Hematological Values Among the Three Monkey Farms

Parameters (units)	Female 4–6 Y			Male 4–6 Y	
	Farm 1 (n=641)	Farm 2 (n=38)	Farm 3 (n=50)	Farm 1 (n=34)	Farm 2 (n=22)
WBC (10^9/L)	15.69 ± 4.27	17.23 ± 4.31a	15.84 ± 4.32	13.23 ± 3.63	17.68 ± 4.65a
RBC (10^{12}/L)	5.57 ± 0.50	6.06 ± 0.53ab	5.38 ± 0.46a	5.57 ± 0.47	6.37 ± 0.52a
HGB (g/L)	132.8 ± 10.31	143.17 ± 11.05ab	125.5 ± 10.70a	136.03 ± 8.33	154.53 ± 8.88a
HCT (%)	44.09 ± 3.43	47.83 ± 3.55ab	42.14 ± 3.53a	45.09 ± 3.27	50.59 ± 2.68a
MCV (fL)	79.16 ± 3.74	78.61 ± 3.32	78.36 ± 4.25	80.43 ± 3.78	79.24 ± 3.89
MCH (pg)	23.95 ± 1.14	23.60 ± 1.06	23.27 ± 1.43a	24.54 ± 1.36	24.43 ± 1.46
MCHC (g/L)	301.77 ± 9.84	299.29 ± 7.15	298.86 ± 11.21a	305.18 ± 13.77	304.59 ± 5.42
PLT (10^9/L)	372.32 ± 89.53	416.26 ± 104.80a	381.00 ± 83.80a	378.81 ± 94.43	365.14 ± 84.55
MPV (fL)	11.74 ± 1.11	11.81 ± 1.09	11.49 ± 1.06	10.93 ± 1.27	11.85 ± 1.08a
PCT (%)	0.44 ± 0.10	0.50 ± 0.10a	0.46 ± 0.10	0.42 ± 0.08	0.43 ± 0.10
NEUT (10^9/L)	8.56 ± 3.91	8.50 ± 2.70	7.64 ± 3.67	6.76 ± 2.68	8.63 ± 3.99
LYMPH (10^9/L)	5.38 ± 2.38	7.13 ± 2.16ab	5.58 ± 2.25	5.12 ± 2.35	6.82 ± 2.18a
MONO (10^9/L)	0.83 ± 0.34	0.91 ± 0.36	0.96 ± 0.32a	0.81 ± 0.37	0.93 ± 0.29
EO (10^9/L)	0.14 ± 0.14	0.13 ± 0.11	0.17 ± 0.16	0.11 ± 0.08	0.24 ± 0.17a
BASO (10^9/L)	0.01 ± 0.01	0.02 ± 0.01ab	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01a
NEUT% (%)	57.69 ± 18.14	48.66 ± 9.63a	51.23 ± 19.35a	56.49 ± 18.40	44.95 ± 16.21a
LYMPH% (%)	35.47 ± 16.51	44.62 ± 9.58a	39.59 ± 17.29	37.11 ± 16.94	48.39 ± 16.07a
MONO% (%)	5.38 ± 2.18	5.75 ± 2.37	6.25 ± 2.25a	5.40 ± 2.02	5.39 ± 1.51
EO% (%)	0.90 ± 0.90	0.84 ± 0.78	1.06 ± 1.05	0.95 ± 0.94	1.20 ± 0.77
BASO% (%)	0.07 ± 0.05	0.11 ± 0.04ab	0.08 ± 0.05	0.06 ± 0.05	0.08 ± 0.04a

a, Significant (P<0.05) difference with respect to farm 1.
b, Significant (P<0.05) difference with respect to farm 3.

Table 3 Continued

Parameters (units)	Female 7–9 Y			Male 7–9 Y		
	Farm 1 (n=597)	Farm 2 (n=17)	Farm 3 (n=47)	Farm 1 (n=51)	Farm 2 (n=8)	Farm 3 (n=23)
WBC (10^9/L)	14.03 ± 4.13	16.45 ± 3.39ab	13.58 ± 4.45	12.80 ± 4.33	16.47 ± 3.45ab	11.98 ± 2.77
RBC (10^{12}/L)	5.57 ± 0.52	6.49 ± 0.48ab	5.57 ± 0.52	5.83 ± 0.42	6.42 ± 0.15ab	5.85 ± 0.54
HGB (g/L)	132.86 ± 10.95	152.00 ± 11.54ab	132.67 ± 9.50	141.00 ± 8.39	160.00 ± 4.65ab	142.71 ± 7.43
HCT (%)	43.91 ± 3.51	50.44 ± 3.73ab	43.70 ± 3.24	45.99 ± 2.84	52.53 ± 2.08ab	46.55 ± 2.85
MCV (fL)	78.88 ± 3.70	75.35 ± 3.20ab	79.06 ± 4.06	78.75 ± 3.60	80.57 ± 4.79	78.90 ± 3.36
MCH (pg)	23.95 ± 1.14	22.55 ± 1.14ab	24.09 ± 1.33	24.28 ± 1.14	24.60 ± 1.27	24.75 ± 1.18
MCHC (g/L)	303.07 ± 9.27	303.93 ± 6.82	303.36 ± 8.59	307.63 ± 6.79	305.62 ± 4.98	309.70 ± 8.65
PLT (10^9/L)	365.59 ± 91.49	402.00 ± 94.67	361.79 ± 90.48	360.34 ± 74.28	444.62 ± 84.64ab	362.48 ± 63.21
MPV (fL)	11.74 ± 1.15	11.60 ± 1.09	11.69 ± 1.18	11.27 ± 1.17	11.39 ± 1.41	11.14 ± 1.11
PCT (%)	0.43 ± 0.09	0.46 ± 0.06	0.42 ± 0.09	0.41 ± 0.08	0.50 ± 0.06ab	0.40 ± 0.06
NEUT (10^9/L)	7.76 ± 3.65	9.14 ± 3.00	7.04 ± 3.59	7.06 ± 3.65	7.90 ± 2.03	7.40 ± 3.13
LYMPH (10^9/L)	4.73 ± 2.07	6.97 ± 2.17ab	5.42 ± 1.90a	4.02 ± 1.90	7.47 ± 2.02ab	3.74 ± 2.15
MONO (10^9/L)	0.79 ± 0.33	0.98 ± 0.31a	0.88 ± 0.37	0.72 ± 0.35	0.90 ± 0.26b	0.57 ± 0.27
EO (10^9/L)	0.14 ± 0.14	0.19 ± 0.15	0.16 ± 0.14	0.14 ± 0.13	0.17 ± 0.10	0.13 ± 0.14
BASO (10^9/L)	0.01 ± 0.01	0.02 ± 0.01ab	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01ab	0.01 ± 0.01
NEUT% (%)	56.95 ± 17.43	54.03 ± 10.41	52.11 ± 15.59	59.52 ± 19.84	48.07 ± 7.12ab	60.95 ± 18.47
LYMPH% (%)	35.50 ± 15.51	38.51 ± 9.53	40.09 ± 13.74	32.69 ± 18.47	45.21 ± 7.36ab	33.06 ± 16.89
MONO% (%)	5.79 ± 2.21	5.65 ± 1.43	6.35 ± 2.10	5.28 ± 2.23	5.52 ± 1.11	4.62 ± 1.98
EO% (%)	1.03 ± 1.03	1.26 ± 1.29	1.24 ± 1.05	1.11 ± 0.95	1.04 ± 0.46	1.13 ± 1.26
BASO% (%)	0.07 ± 0.05	0.11 ± 0.04ab	0.07 ± 0.05	0.04 ± 0.05	0.11 ± 0.04ab	0.04 ± 0.05

a, Significant (P<0.05) difference with respect to farm 1.
b, Significant (P<0.05) difference with respect to farm 3.

Table 4 Comparison of Biochemical Values Among the Three Monkey Farms

Parameters (units)	Female 4–6 Y			Male 4–6 Y	
	Farm 1 (n=641)	Farm 2 (n=38)	Farm 3 (n=50)	Farm 1 (n=34)	Farm 2 (n=22)
ALT (U/L)	26.47 ± 17.87	26.75 ± 12.61	26.30 ± 16.37	29.44 ± 17.66	22.14 ± 9.81
AST (U/L)	52.29 ± 14.33	37.55 ± 11.67ab	54.09 ± 14.76	52.53 ± 14.33	38.48 ± 8.90a
TP (g/L)	76.63 ± 7.12	70.22 ± 5.35ab	78.47 ± 6.94	78.44 ± 4.73	69.79 ± 5.42a
ALB (g/L)	41.38 ± 5.88	38.93 ± 3.45ab	42.09 ± 5.78	45.74 ± 4.24	39.32 ± 3.48a
TBIL (μmol/L)	5.49 ± 1.55	1.59 ± 1.20ab	4.78 ± 1.88a	5.59 ± 2.01	1.33 ± 0.66a
D-BIL (μmol/L)	1.50 ± 0.68	1.02 ± 0.44ab	1.37 ± 0.67	1.36 ± 0.55	0.99 ± 0.43a
ALP (U/L)	191.96 ± 80.80	194.39 ± 73.49b	352.30 ± 147.87a	233.88 ± 119.74	291.40 ± 215.09
GLU (mmol/L)	3.92 ± 1.73	5.68 ± 1.37ab	4.20 ± 1.81	3.04 ± 0.98	6.11 ± 1.15a
BUN (mmol/L)	6.62 ± 1.42	7.55 ± 1.51a	7.23 ± 1.61a	6.70 ± 1.39	7.61 ± 1.11a
CREA (μmol/L)	77.35 ± 13.73	60.29 ± 11.37ab	71.58 ± 18.37a	95.96 ± 13.41	91.10 ± 15.29
Ca (mmol/L)	2.49 ± 0.14	2.49 ± 0.11	2.49 ± 0.14	2.56 ± 0.12	2.43 ± 0.10a
P (mmol/L)	1.59 ± 0.34	1.47 ± 0.28ab	1.66 ± 0.35	1.44 ± 0.30	1.65 ± 0.31a
CHOL (mmol/L)	2.79 ± 1.04	2.79 ± 0.93	3.10 ± 0.86a	2.73 ± 0.67	2.42 ± 0.52
TG (mmol/L)	0.55 ± 0.21	0.85 ± 0.40ab	0.64 ± 0.41	0.46 ± 0.18	0.91 ± 0.49a
HDL-C (mmol/L)	1.41 ± 0.54	1.31 ± 0.46b	1.54 ± 0.42a	1.52 ± 0.26	1.21 ± 0.27a
LDL-C (mmol/L)	1.28 ± 0.55	1.41 ± 0.49	1.40 ± 0.57	1.15 ± 0.42	1.22 ± 0.36
LDH (U/L)	474.06 ± 213.51	390.26 ± 77.54ab	499.66 ± 193.31	547.97 ± 252.03	360.70 ± 74.49a

a, Significant (P<0.05) difference with respect to farm 1.
b, Significant (P<0.05) difference with respect to farm 3.

Table 4 Continued

Parameters (units)	Female 7–9 Y			Male 7–9 Y		
	Farm 1 (n=597)	Farm 2 (n=17)	Farm 3 (n=47)	Farm 1 (n=51)	Farm 2 (n=8)	Farm 3 (n=23)
ALT (U/L)	27.23 ± 18.36	21.53 ± 14.65	29.46 ± 17.22	26.25 ± 18.74	32.75 ± 11.11	25.52 ± 17.30
AST (U/L)	47.36 ± 13.25	39.62 ± 12.99a	44.29 ± 12.57	48.83 ± 12.69	42.50 ± 9.75	49.87 ± 15.66
TP (g/L)	77.59 ± 6.61	71.29 ± 5.94a	73.43 ± 6.56a	79.72 ± 4.97	72.25 ± 5.52ab	79.01 ± 3.75
ALB (g/L)	42.26 ± 5.31	38.25 ± 4.07a	39.06 ± 5.41a	46.57 ± 3.46	41.16 ± 3.96ab	46.95 ± 1.89
TBIL (μmol/L)	5.27 ± 1.61	1.46 ± 1.02ab	5.65 ± 1.63	5.42 ± 1.45	1.54 ± 0.90ab	5.22 ± 1.82
D-BIL (μmol/L)	1.44 ± 0.63	0.94 ± 0.41ab	1.45 ± 0.52	1.55 ± 0.71	1.20 ± 0.37a	1.45 ± 0.65
ALP (U/L)	160.56 ± 56.37	151.13 ± 48.75b	74.07 ± 9.01a	164.44 ± 45.08	116.12 ± 30.12ab	89.09 ± 15.32a
GLU (mmol/L)	4.11 ± 1.70	5.37 ± 0.86ab	4.39 ± 1.83	3.36 ± 1.35	4.66 ± 0.89ab	3.55 ± 0.94
BUN (mmol/L)	6.45 ± 1.49	7.19 ± 1.72b	6.06 ± 1.47	7.09 ± 1.20	7.77 ± 1.93b	6.47 ± 1.32
CREA (μmol/L)	77.30 ± 14.12	77.00 ± 16.78	75.20 ± 14.97	104.43 ± 12.98	94.29 ± 16.04b	108.21 ± 10.22
Ca (mmol/L)	2.50 ± 0.15	2.48 ± 0.12	2.45 ± 0.16	2.57 ± 0.10	2.51 ± 0.11	2.54 ± 0.13
P (mmol/L)	1.47 ± 0.32	1.34 ± 0.24	1.39 ± 0.26	1.48 ± 0.29	1.49 ± 0.18	1.51 ± 0.23
CHOL (mmol/L)	3.06 ± 0.99	3.18 ± 0.74b	2.27 ± 0.81a	3.09 ± 0.63	2.69 ± 0.56	2.83 ± 0.68
TG (mmol/L)	0.71 ± 0.36	0.88 ± 0.45b	0.58 ± 0.32a	0.60 ± 0.43	1.43 ± 0.86ab	0.66 ± 0.39
HDL-C (mmol/L)	1.57 ± 0.51	1.47 ± 0.26b	1.19 ± 0.49a	1.66 ± 0.36	1.27 ± 0.13ab	1.58 ± 0.39
LDL-C (mmol/L)	1.35 ± 0.57	1.63 ± 0.54b	1.02 ± 0.40a	1.31 ± 0.50	1.41 ± 0.45	1.13 ± 0.42
LDH (U/L)	468.18 ± 219.13	374.20 ± 89.39ab	499.05 ± 213.03	435.41 ± 222.26	381.62 ± 90.03	386.63 ± 197.66

a, Significant (P<0.05) difference with respect to farm 1.
b, Significant (P<0.05) difference with respect to farm 3.

observed in MCHC, MPV, NEUT, EO, MONO%, and EO% values among the three farms (P>0.05). No statistically significant difference was found in ALT values among the three farms in both age groups (P>0.05). In the 4–6 Y age group, no significant difference was observed in Ca values in females among the three farms (P>0.05), whereas a significant difference was found in males (P<0.05). In the 7–9 Y age group, no significant differences were observed in the Ca and P values in males and females among the three farms (P>0.05).

Correlation of hematological and biochemical parameters with age

The correlation analysis of hematological and biochemical parameters with age is presented in **Table 5**. WBC, RBC, HGB, HCT, LYMPH, LYMPH%, ALP, GLU, BUN, TG, and LDH values were significantly higher in the 1–3 Y group than the other age groups. RBC and ALP levels were generally higher in males than in females, and the difference gradually decreased with age (**Figure 1**). The serum TP and

Table 5 Correlation of All Parameters with Age

Parameters	P-value					
	Age 1–3 Y	Age 4–6 Y	Age 7–9 Y	Age 10–12 Y	Age 12–15 Y	Age above 15 Y
WBC	NS	<0.01	NS	<0.05	NS	NS
RBC	<0.01	NS	NS	<0.01	NS	<0.05
HGB	<0.01	NS	NS	NS	NS	<0.01
HCT	<0.01	NS	NS	NS	NS	<0.01
MCV	<0.01	<0.01	NS	<0.05	NS	NS
MCH	<0.01	NS	NS	<0.01	NS	NS
MCHC	NS	<0.01	NS	NS	NS	<0.05
PLT	NS	<0.01	<0.01	NS	NS	<0.05
MPV	<0.05	NS	<0.01	NS	NS	NS
PCT	NS	<0.01	<0.01	NS	NS	<0.05
NEUT	<0.01	NS	NS	<0.01	NS	NS
LYMPH	<0.01	<0.01	NS	NS	<0.05	NS
MONO	NS	<0.01	NS	NS	NS	<0.01
EO	NS	<0.01	NS	<0.01	NS	NS
BASO	<0.01	<0.01	NS	NS	NS	NS
NEUT% (%)	<0.01	<0.01	NS	<0.01	NS	NS
LYMPH% (%)	<0.01	<0.01	NS	<0.01	NS	NS
MONO% (%)	<0.01	<0.05	NS	<0.05	<0.05	NS
EO% (%)	NS	<0.01	NS	<0.01	NS	NS
BASO% (%)	<0.01	NS	<0.05	NS	NS	NS
ALT	NS	<0.01	<0.01	NS	NS	NS
AST	NS	<0.01	<0.01	NS	NS	NS
TP	<0.01	<0.05	NS	<0.05	NS	NS
ALB	NS	NS	<0.01	NS	<0.05	NS
TBIL	<0.01	<0.01	<0.05	NS	NS	NS
D-BIL	<0.01	<0.01	NS	<0.05	NS	<0.01
ALP	<0.01	<0.01	NS	NS	NS	NS
GLU	<0.01	NS	NS	<0.01	NS	<0.01
BUN	<0.01	<0.01	NS	NS	<0.05	NS
CREA	<0.01	<0.01	<0.01	<0.05	NS	NS
Ca	NS	NS	<0.01	NS	NS	<0.01
P	<0.01	<0.01	NS	<0.01	NS	NS
CHOL	<0.01	<0.01	NS	NS	NS	NS
TG	<0.01	NS	<0.01	<0.05	NS	<0.05
HDL-C	NS	<0.01	<0.05	NS	NS	NS
LDL-C	<0.01	NS	NS	NS	NS	NS
LDH	NS	<0.01	<0.01	NS	NS	<0.01

*NS, not significant.

NEUT% increased with age in the 1–3 Y and 4–6 Y groups, and remained stable thereafter. Moreover, LYMPH% showed an opposite trend from NEUT%.

Discussion

Our study provided a reference database of hematologic and biochemical parameters in 4,834 healthy cynomolgus monkeys of a range of ages, and determined the effects of age, sex, and colony conditions on these parameters. Cynomolgus monkeys, the most commonly used non-human primate experimental animals, substantially contribute to many biomedical research fields [12]. The goal of our study was to establish the reference ranges of hematologic and biochemical parameters in healthy cynomolgus monkeys for evaluating healthy individuals, constructing disease models, improving the effectiveness of disease diagnosis and treatment, and evaluating drug safety in non-clinical research. Moreover, we provide data on old age groups not reported in previous studies. However, the results were affected by factors including housing [16], feeding, fasting, and sedation.

Animals in our study were housed indoors in mixed-sex social groups in large cages, but under laboratory conditions, they were usually transferred into individual cages providing a relatively small space. This change in living environment might have caused stress, which consequently directly influenced the sympathetic adrenal medulla system and potentially led to the production of stress hormones, such as epinephrine, that increased blood parameters (e.g., WBC).

Previous research has reported the differences in blood hematologic and biochemical measurements according to age and sex [7]. For example, hematologic and biochemical parameters including RBC, HGB, HCT, ALP, and CREA

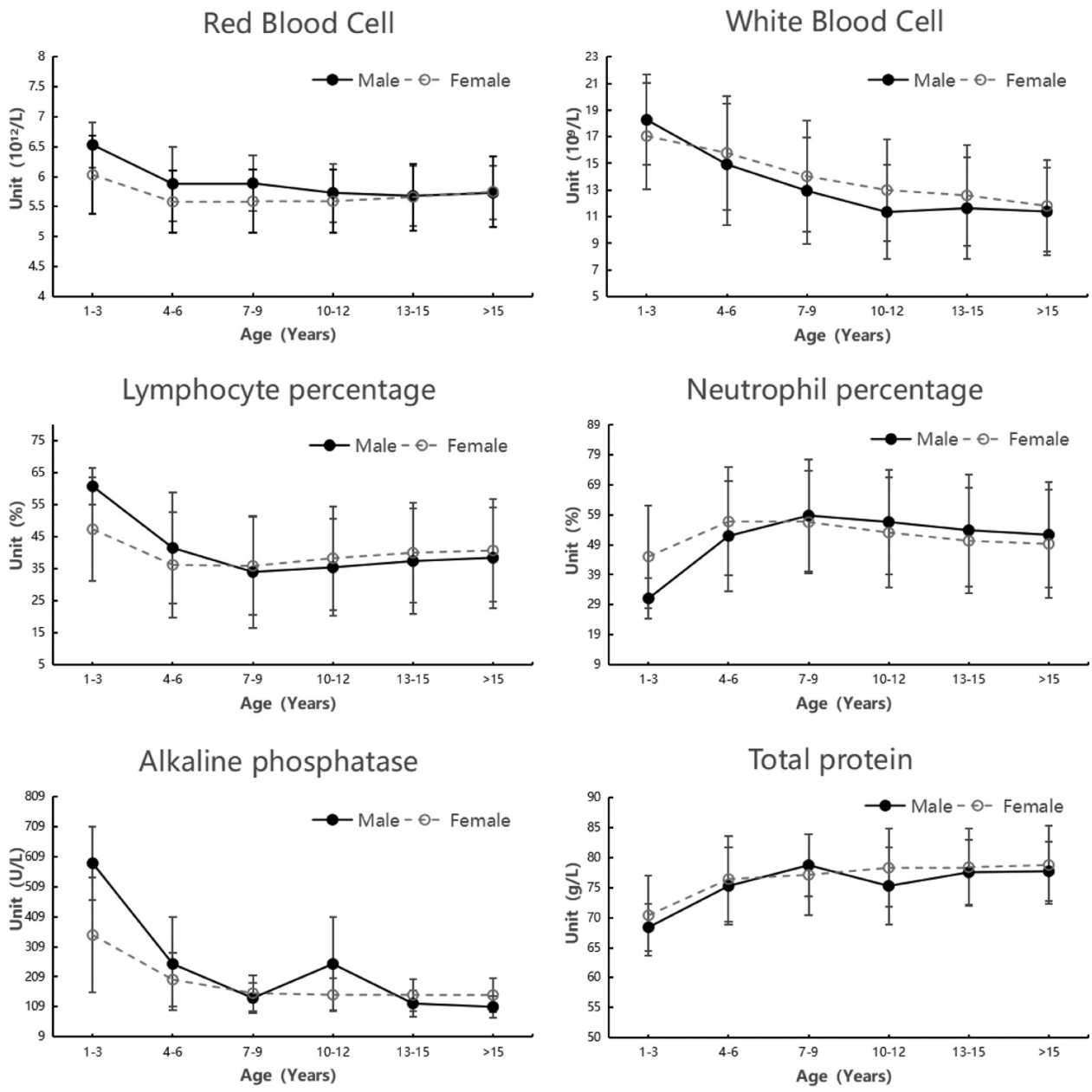

Figure 1 Changes in red blood cell count, white blood cell count, lymphocyte percentage, neutrophil percentage, alkaline phosphatase, and total protein in males and females with age.

have shown significant effects dependent on both age and sex, in agreement with our statistical results. In addition, WBC, HGB, and MCHC displayed high variability within the age range from 1 to 5 Y, and the values were significantly higher in this group than in other groups. Some concentrations of serum biochemical parameters were significantly lower within the age range from 1 to 5 Y, such as ALT, ALP, LDH, and TP, whereas the concentration of TBIL was significantly higher within the age range from 3 to 5 Y, possibly because of the environmental and breeding conditions.

Because geographical location, breeding conditions, and climatic factors may affect hematological and biochemical values, we compared and analyzed the variations in the results among the three monkey farms, and found significant differences in hematological parameters, including RBC, HGB, and HCT values. The three monkey farms in our study are located in Kunming and Guangzhou in China. Kunming, in a temperate climatic zone, is located in the central Yunnan-Guizhou Plateau, southwest China (102°10' to 103°40' E, 24°23' to 26°22' N), where altitudes range from 1500 m to 2800 m. Guangzhou, in a subtropical climate zone, is located in south China (112°57' to 114°3' E, 22°26' to 23°56' N), where altitudes range from 0 m to 1210 m. In the present study, the results (**Table 3**) demonstrated that the WBC, RBC, HGB, LYMPH, and LYMPH% values were significantly higher, whereas the NEUT% values were lower, in the Kunming animals than the Guangzhou animals, thus supporting the hypothesis that adaptation to lower oxygen at high altitude results in a physiological increase in RBC and hemoglobin concentration [17].

A comparison of the ALP and LDH values among age groups indicated that the concentrations of ALP and LDH in juveniles were significantly higher than those in other age groups. The ALP level decreased with age, then reached a stable level in

adults. ALP is produced in the liver, bones, small intestine, and placenta, and usually reflects bone anabolism [18, 19]. It may be associated with elevated osteoblast activity in juvenile monkeys. A similar change in ALP level has also been reported by Ogawa [20]. Previous studies have indicated that ALP is significantly higher in patients with metastatic prostatic cancer, and may serve as a prognostic marker of bone metastases and multiple osteoblastic lesions. [21] Therefore, detecting ALP levels is meaningful in diagnosing and monitoring bone diseases, as well as evaluating drug efficacy. Although serum LDH levels are known to increase under myocardial injury and liver injury, we observed significantly higher LDH in the juvenile monkeys than in the other age groups, in agreement with a report by Koga et al. [22]. Whether this finding was associated with high levels of aerobic metabolism in juvenile monkeys remains to be further studied.

In conclusion, reference ranges for healthy cynomolgus monkeys were established according to age and sex in this study. Researchers and laboratory technicians routinely need to identify etiological factors, improve clinical care, and establish human disease models and new drug screening. However, multiple uncontrollable factors, including differences between individuals and breeding environments, affect hematologic and biochemical values, thus resulting in differences in data between laboratories. The above factors should therefore be considered in practical applications.

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