Reference data of clinical chemistry, haematology and blood coagulation parameters in juvenile cynomolgus monkeys (*Macaca fascicularis*)

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ABSTRACT: Juvenile cynomolgus monkeys are valuable models for studying human diseases. Reference data of clinical chemistry, haematology and blood coagulation parameters of juvenile cynomolgus monkeys are very important for clinical diagnosis and conducting research. In this study, 72 blood samples (obtained from 35 males and 37 females) and 20 blood samples (obtained from 10 males and 10 females) were used to determine normal data of clinical serum chemistry, haematological profiles and normal blood coagulation parameters in juvenile cynomolgus monkeys. Seventeen markers of clinical serum chemistry, twenty-nine markers of haematology and two parameters of blood coagulation were analysed. These data may provide valuable information for veterinarians and investigators using juvenile cynomolgus monkeys in research on disease treatment and in experimental studies.

Keywords: serum chemical parameters; complete blood count; prothrombin time; activated partial thromboplastin time; juvenile *Macaca fascicularis*

Non-human primates play an important role in research into human disease because of their close genetic relationship to humans. Many previous studies have reported clinical chemistry and haematological data in cynomolgus, rhesus and squirrel monkeys caught in the wild (Kaplan 1977; Matsuzawa and Nagai 1994; Andrade et al. 2004). In recent years, purpose-bred juvenile cynomolgus monkeys have become commercially available. Normal clinical chemical, haematological and blood coagulation parameters are important complements to various scientific investigations. These data are crucial for the diagnosis and treatment of sick animals and for improving therapies and experimentation. Such data have been reported from different research groups, and have been obtained under a variety of environmental and experimental conditions. It is also frequently unclear whether the same testing methods were used throughout the data collection period.

The values obtained in this study will be used as baseline data for clinical chemistry, haematology and blood coagulation parameters for healthy juvenile cynomolgus monkeys.

MATERIAL AND METHODS

Animals

The juvenile cynomolgus monkeys used in this study were provided by Kunming Biomed International (KBI). All of the animals were housed individually in stainless-steel cages (length × width × height: 80cm × 80cm × 80cm).

Seventy-two healthy juvenile cynomolgus monkeys (35 males and 37 females) were used for clinical chemistry and haematology tests, and 20 healthy juvenile cynomolgus monkeys (10 males and 10 females) were used for coagulation tests, respectively. These animals were from 2.5 to 3.5 years old with body weights of between 2.88 kg and 3.7 kg. They were free of specific pathogenic microorganisms such as Salmonella, Pathogenic dermal

fungi, Shigella, *Mycobacterium tuberculosis* and *Cercopithecine herpesvirus* Type I, according to the National Standards of Experimental Animals in China.

All animals were maintained in controlled animal rooms at temperatures of between 18 °C to 26 °C and relative humidity of 30% to 70%. Twelve hours (from 8:00 to 20:00) of continuous fluorescent lighting were provided daily. Animal were fed twice a day with commercial monkey chew (crude protein 21.5%, and crude fat 6.8%), supplemented with fresh fruits and vegetables once a day. Animal were given free access to tap water, supplied by an automatic watering system. All experimental procedures were approved in advance by the Institutional Animal Care and Use Committee (IACUC) of KBI. Furthermore, the animal facility is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Blood collection

Blood samples were obtained from the femoral vein of a non-anaesthetised monkey using a 2.5 ml syringe with 22 gauge needle between 9:00 and 10:30 a.m. after fasting for 16 hours. Different anti-coagulant reagents were used in this study, 2 ml complete blood without anti-coagulants were used for clinical chemistry examinations, 1 ml complete blood with ethylenediamine tetraacetic acid-potassium (EDTA-K_2) for complete blood count examinations, whereas, 2.7 ml complete blood with trisodium citrate(1 : 9) was used for coagulation tests. The serum was separated by centrifugation at 3000 rpm for 15 min for clinical chemistry examination and the plasma was separated by centrifugation at 2500 rpm for 10 min for coagulation examination.

Analysis and statistical evaluation

Clinical chemistry measurements were carried out on serum samples using Abbott CI 16200 (Abbott Laboratories, Pasadena, CA, USA) equipment. Analysis included lactate dehydrogenase (LDH, U/l), aspartate aminotransferase (AST, U/l), alanine aminotransferase (ALT, U/l), alkaline phosphatase (AKP, U/l), total protein (TP, g/l), albumin (ALB, g/l), globulin (GLOB, g/l), albumin/globulin(A/G), cholesterol (CHOL, mmol/l), triglyceride (TG, mmol/l), glucose (GLU, mmol/l), blood urea nitrogen (BUN, mmol/l), creatinine (CREA, μmol/l), chloride (Cl, mmol/l), kalium(K, mmol/l), calcium (Ca, mmol/l) and phosphate (P, mmol/l).

Complete blood count examinations were performed on the whole blood with EDTA-K_2 as an anti-coagulant using Sysmex Hemacytometer XT-2000i (Kobe, Japan). Analysis included white blood cell count (WBC, 10^9/l), neutrophile count (NEUT, 10^9/l and percents), lymphocyte count (LYMPH, 10^9/l and percents), monocyte count (MONO, 10^9/l and percents), eosinophil count (EO, 10^9/l and percents), and basophil count (BASO, 10^9/l and percents), red blood cell count (RBC, 10^{12}/l), hemoglobin (HGB, g/l), hematocrit (HCT, percents), mean corpusular volume (MCV, fl), mean corpusular hemoglobin (MCH, pg), mean corpusular hemoglobin concerntration (MCHC, g/l), blood platelet count (PLT, 10^9/l), red blood cell distribution width-standard deviation (RDW-SD, fl), red cell distribution width coefficient of variability (RDW-CV, percents), platelet distribution width (PDW, fl), mean platelet volume (MPV, fl), plateletocrit (PCT, percents), reticulocyte (RET, 10^9/l and percents), immature reticulocyte fraction (IRF, percents), low fluorescent reticulocyte (LFR, percents), middle fluorescent reticulocyte (MFR, percents) and high fluorescent reticulocyte (HFR, percents).

Blood coagulation function was determined on plasma samples separated from blood using trisodium citrate as an anti-coagulant. Prothrombin times (PT) and activated partial thromboplastin times (APTT) were determined using a STAGO-STA autoanalyzer (Paris, France).

Statistical analysis

All data were expressed as means ± SD. Subsequently, independent-sample *t*-tests were performed to identify significant differences between the data of the males and the females. An analysis of variance (ANOVA) was performed for clinical chemistry, haematology and haematology parameters, with sex as a factor and body weight as covariate. Statistical significance was reached in the case of $P < 0.05$ (difference significant) or $P < 0.01$(very significant difference). SPSS (Statistical Package for Social Science) 11.5 statistical software (Chicago, IL, USA) was used for data evaluation.

RESULTS

Clinical chemistry data

The baseline data for clinical chemistry parameters collected from 72 juvenile cynomolgus monkeys (35 males and 37 females) are shown in Table 1. The mean AKP, ALB, A/G, GLU, CREA and P in male animals were significant higher than those in female animals. The most remarkable differences were presented in AKP (male vs. female, $P < 0.01$), ALB (male vs. female, $P < 0.01$), A/G (male vs. female, $P < 0.01$), CREA (male vs. female, $P < 0.01$), P (male vs. female, $P < 0.01$). A significant effect of body weight was found for ALT, GLU and CREA ($P = 0.013$, 0.033 and 0.0003, respectively). The scatter plots for ALT, GLU and CREA are presented in Figure 1. The data for ALT, GLU and CREA showed an increase with increasing body weight.

Complete blood count

The normal data of complete blood count of 72 juvenile cynomolgus monkeys (35 males and 37 females) are presented in the Table 2. Statistical differences between sexes were observed in RBC, RET, IRF, LFR and MFR. RBC (male vs. female, $P < 0.05$), IRF (male vs. female, $P < 0.05$), LFR (male vs. female, $P < 0.05$). The most remarkable differences were noted in RET (male vs. female, $P < 0.01$) and MFR (male vs. female, $P < 0.01$).

No significant effect of body weight as a covariate was observed for any of the parameters from Table 2.

Blood coagulation parameters

The normal data of coagulation parameters in juvenile cynomolgus monkeys are presented in Table 3. No significant differences in PT and APTT

Table 1. Normal clinical chemistry data in healthy juvenile cynomolgus monkeys

Parameter(unit)	Males and females ($n = 72$)	Males ($n = 35$)	Females ($n = 37$)	Body weight effect
LDH (U/l)	604.72 ± 218.04	572.86 ± 201.61	634.86 ± 231.20	ns
AST (U/l)	50.60 ± 22.63	47.09 ± 15.25	53.92 ± 27.70	ns
ALT (U/l)	48.06 ± 20.00	46.77 ± 17.50	49.27 ± 22.29	0.013
AKP (U/l)	460.78 ± 161.30	524.83 ± 155.61**	400.19 ± 143.83	ns
TP (g/l)	81.49 ± 4.16	82.03 ± 4.27	80.97 ± 4.03	ns
ALB (g/l)	43.72 ± 3.39	45.11 ± 2.82**	42.41 ± 3.39	ns
GLOB (g/l)	37.76 ± 3.74	36.91 ± 3.73	38.57 ± 3.63	ns
A/G	1.17 ± 0.17	1.24 ± 0.16**	1.11 ± 0.15	ns
CHOL (mmol/l)	3.28 ± 0.67	3.30 ± 0.70	3.25 ± 0.65	ns
TG (mmol/l)	0.32 ± 0.17	0.31 ± 0.15	0.34 ± 0.19	ns
GLU (mmol/l)	3.49 ± 1.05	3.59 ± 1.05*	3.39 ± 1.05	0.033
BUN (mmol/l)	8.19 ± 1.44	8.24 ± 1.47	8.15 ± 1.44	ns
CREA (μmol/l)	63.82 ± 9.31	68.09 ± 8.69**	59.78 ± 8.08	0.0003
Cl (mmol/l)	109.71 ± 2.86	109.17 ± 2.60	110.22 ± 3.04	ns
K (mmol/l)	5.05 ± 0.49	5.01 ± 0.38	5.08 ± 0.57	ns
Ca (mmol/l)	2.62 ± 0.13	2.64 ± 0.10	2.60 ± 0.15	ns
P (mmol/l)	2.27 ± 0.39	2.48 ± 0.34**	2.06 ± 0.32	ns

Data presented are arithmetic mean data ± SD. The statistical significance is indicated with ** or * in the column 'males' when comparing data of male monkeys with female monkeys (*$P < 0.05$, **$P < 0.01$). For the covariate body weight, the statistical significance is given in the column 'body weight effect' (ns = not significant)

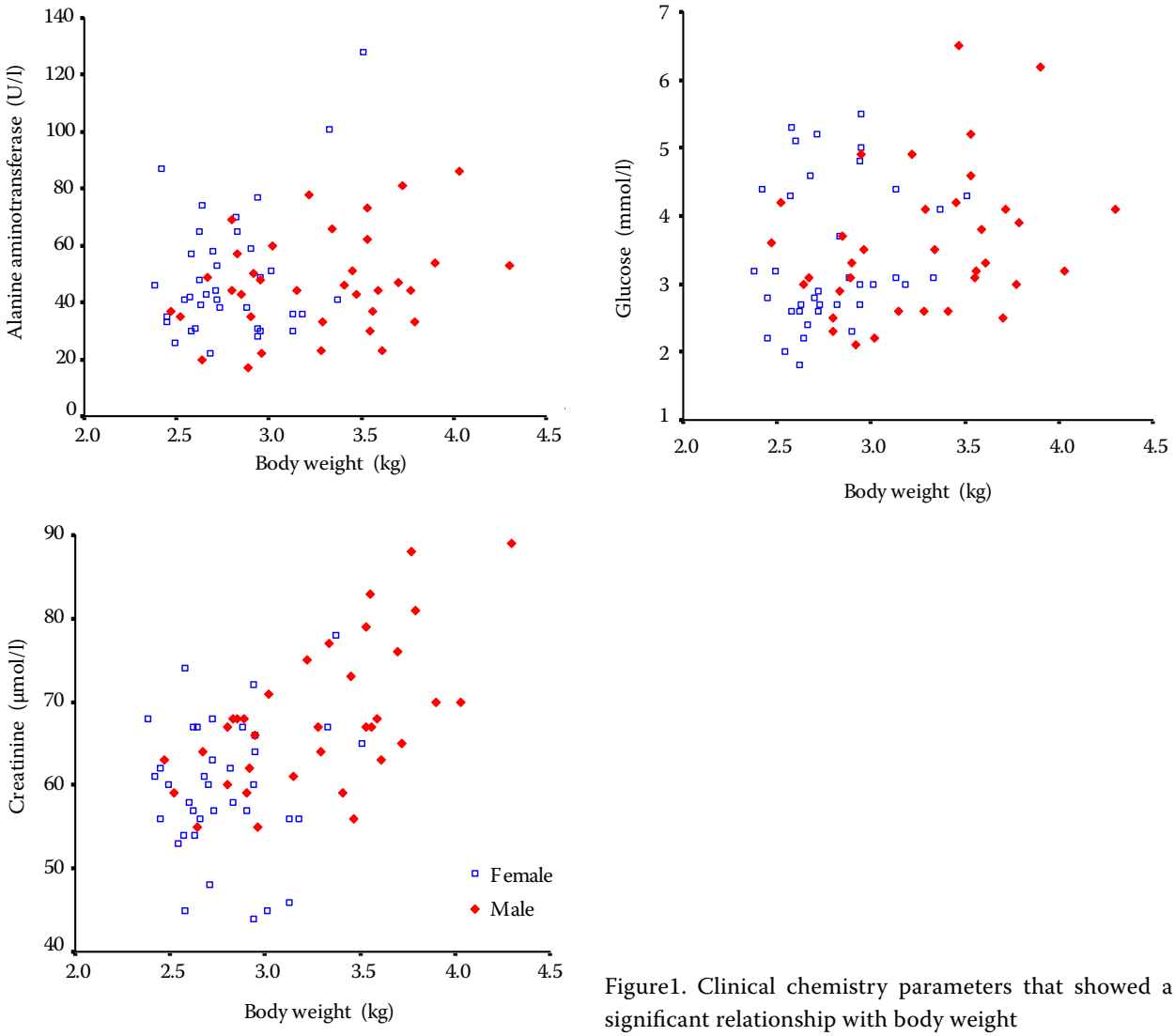

Figure1. Clinical chemistry parameters that showed a significant relationship with body weight

were observed between male with female juvenile cynomolgus monkeys.

DISCUSSION

Non-human primates, especially rhesus monkeys and cynomolgus monkeys are very important models for studies on human diseases and for biological research in general. Here, we reported the reference data of clinical chemical, haematological, and blood coagulation parameters of juvenile cynomolgus monkeys. These normal data are indispensable reference data for disease treatment, animal experiment design and pharmacodynamics appraisement.

The most significant difference between our study and others was observed in the clinical chemistry data of the non-human primates, our study showed the TP (81.49 g/l) value higher than the 75 g/l reported from four year-old cynomolgus monkeys from the Philippines (Matsuzawa and Nagai 1994). Schuurman and Smith (2005) reported TP values (89.1 g/l) of cynomolgus monkeys from USA to be higher still than the value of 81.49 g/l and 75 g/l, while the LDH value (604.72 U/l) reported in our study is enormously higher than the value of 340 U/l reported by these authors (Schuurman and Smith 2005), These differences may be due to several reasons including deficiency of vitamins (Kaplan 1977), unrecognised disease (Melville et al. 1967), housing and stress conditions, and method of handling and blood collection (Andrade et al. 2004). Some previous studies have reported the reference data of haematology parameters in baboons, cynomolgus monkeys and rhesus monkeys (Matsuzawa and Nagai 1994; Schuurman

Table 2. Complete blood count of juvenile cynomolgus monkeys

Parameter (unit)	Males and females (n = 72)	Males (n = 35)	Females (n = 37)	Body weight effect
WBC (10^9/l)	13.31 ± 4.41	12.42 ± 3.77	14.16 ± 4.84	ns
RBC (10^{12}/l)	6.22 ± 0.45	6.34 ± 0.51*	6.11 ± 0.36	ns
HGB (g/l)	141.88 ± 10.53	144.57 ± 9.67	139.32 ± 10.81	ns
HCT (%)	48.98 ± 2.94	49.73 ± 3.01	48.28 ± 2.72	ns
MCV (fl)	78.84 ± 3.34	78.59 ± 3.62	79.07 ± 3.08	ns
MCH (pg)	22.81 ± 1.11	22.83 ± 1.05	22.79 ± 1.18	ns
MCHC (g/l)	289.49 ± 10.46	290.71 ± 8.70	288.32 ± 11.89	ns
PLT (10^9/l)	382.13 ± 91.97	373.51 ± 81.03	390.27 ± 101.70	ns
RDW-SD (fl)	41.58 ± 2.97	41.15 ± 2.46	41.97 ± 3.36	ns
RDW-CV (%)	14.75 ± 1.28	14.66 ± 1.14	14.83 ± 1.40	ns
PDW (fl)	14.02 ± 2.38	13.88 ± 2.19	14.15 ± 2.57	ns
MPV (fl)	12.00 ± 1.16	11.94 ± 1.05	12.06 ± 1.26	ns
PCT (%)	0.45 ± 0.09	0.44 ± 0.09	0.46 ± 0.09	ns
NEUT (10^9/l)	6.43 ± 3.72	5.83 ± 3.13	7.00 ± 4.16	ns
NEUT (%)	46.66 ± 14.37	45.36 ± 12.86	47.89 ± 15.75	ns
LYMPH (10^9/l)	6.00 ± 2.28	5.88 ± 2.21	6.12 ± 2.37	ns
LYMPH (%)	45.96 ± 13.20	47.52 ± 12.25	44.49 ± 14.05	ns
MONO (10^9/l)	0.89 ± 0.59	0.78 ± 0.39	0.98 ± 0.72	ns
MONO (%)	6.43 ± 2.86	6.21 ± 2.18	6.63 ± 3.40	ns
EO (10^9/l)	0.09 ± 0.10	0.08 ± 0.09	0.10 ± 0.10	ns
EO (%)	0.78 ± 1.06	0.76 ± 1.22	0.79 ± 0.91	ns
BASO (10^9/l)	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.03	ns
BASO (%)	0.17 ± 0.11	0.15 ± 0.10	0.19 ± 0.12	ns
RET (%)	1.15 ± 0.43	1.00 ± 0.30**	1.29 ± 0.49	ns
RET (10^9/l)	71.19 ± 27.07	62.89 ± 17.75**	79.05 ± 31.89	ns
IRF (%)	14.54 ± 5.41	13.11 ± 4.97*	15.89 ± 5.52	ns
LFR (%)	85.46 ± 5.41	86.89 ± 4.97*	84.11 ± 5.52	ns
MFR (%)	8.73 ± 4.17	7.31 ± 3.83**	10.08 ± 4.08	ns
HFR (%)	5.81 ± 3.24	5.80 ± 3.32	5.81 ± 3.22	ns

Data presented are arithmetic mean data ± SD. The statistical significance is indicated with ** or * in the column 'males' when comparing data of male monkeys with female monkeys (*P < 0.05, **P < 0.01). For the covariate body weight, the statistical significance is given in the column 'body weight effect' (ns = not significant)

Table 3. Blood coagulation parameters of juvenile cynomolgus monkeys

Parameter (unit)	Males and females (n = 20)	Males (n = 10)	Females (n = 10)
PT (s)	11.89 ± 0.63	12.18 ± 0.73	11.60 ± 0.37
APTT (s)	28.23 ± 2.11	29.22 ± 2.08	27.24 ± 1.81

et al. 2004; Schuurman and Smith 2005; Chen et al. 2009). There were some differences in the haematology parameters reported previously and the parameters of the juvenile cynomolgus monkeys in our study. Furthermore, when interpreting our results it should be noted that the blood samples were collected without anaesthesia. Thus, the animals were under stress at blood collection, which possibly interfered with the levels of some parameters sensitive to stress. Ives and Dack (1956) reported the acute stress caused in animals not under anaesthesia in response to handling, resulting in the so-called' alarm reaction' characterised by haemoconcentration, lymphocytosis and neutrophilia.

When comparing our results with previous studies on haematology and clinical chemistry parameters (Matsuzawa and Nagai 1994; Schuurman et al. 2004; Schuurman and Smith 2005; Chen et al. 2009), we conclude that the long-term nutrition condition, climate, the home, genetic background and age of the animals could affect the clinical chemistry and haematology parameters.

Moreover, many of the chemistry and haematology parameters, in particular the coagulation parameters established in this study, have yet to be reported in baboons and cycomolgus monkeys. Our results will thus provide valuable supplements to the data on coagulation parameters of juvenile cynomolgus monkeys. Chen et al. (2009) reported a PT of 14.18 s and APTT of 43.04 s in rhesus monkeys, but in our study we observed a PT of 11.89 s and APTT of 28.23 s in juvenile cynomolgus monkeys. Thus, it appears that there exist great differences in PT and APTT between juvenile cynomolgus monkeys and rhesus monkeys. Regarding the effect of age, as only a relatively small age range of juvenile cynomolgus monkeys (2.5 to 3.5 years old) was sampled in this study, a larger sample size is needed to confirm the significant effect of age on PT and APTT in cynomolgus monkeys.

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