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Reference values of clinical chemistry and hematology parameters in rhesus monkeys (*Macaca mulatta*)

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Abstract: Background: Rhesus monkey models are valuable to the studies of human biology. Reference values for clinical chemistry and hematology parameters of rhesus monkeys are required for proper data interpretation.
Methods: Whole blood was collected from 36 healthy Chinese rhesus monkeys (*Macaca mulatta*) of either sex, 3 to 5 yr old. Routine chemistry and hematology parameters, and some special coagulation parameters including thromboelastograph and activities of coagulation factors were tested.
Results and conclusion: We presented here the baseline values of clinical chemistry and hematology parameters in normal Chinese rhesus monkeys. These data may provide valuable information for veterinarians and investigators using rhesus monkeys in experimental studies.

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Key words: chemistry – coagulation – hematology – reference values – rhesus monkey

Abbreviations: TBA, total serum bile acids; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AST/ALT, alanine aminotransferase/aspartate aminotransferase ratio; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; CK, creatine kinase; LDH, lactate dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; PT, prothrombin times; APTT, activated partial thromboplastin times; TEG, thromboelastograph; R, reaction time; MA, maximum amplitude; CI, coagulation index.

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Introduction

As the closest phylogenetic relatives to humans, non-human primates have played an indispensable role in biomedical researches. These animals are often the best—and sometimes the only—available model for studying a variety of human health issues, ranging from diseases and disorders to potential therapies and preventive strategies [1]. Because there are so many hurdles in xenotransplantation, its clinical practices are hard to promote. Xenotransplantation from pigs to non-human primates has made great progresses in this field.

As an Old World Monkey, rhesus monkey (*Macaca mulatta*) is one of our closest relatives outside of the great apes—chimpanzees, gorillas, and orangutans—making it highly informative on

many levels and widely used in the pre-clinical biomedical researches, including transplantation [2]. Given the current shortage of Indian-origin rhesus, many researchers have turned to rhesus macaques from China as a substitute, whereas, some differences have been identified between these two groups [3]. Understanding the characterizations of Chinese rhesus monkey is required for the proper data interpretation in animal studies using these monkeys.

Clinical chemistry and hematology parameters have closely paralleled the course of diseases and have been considered as the important routine references for diagnoses and therapy monitoring. Analogously, the baseline values of chemistry and hematology parameters of rhesus monkey are valuable to distinguish the pathological changes in these animal models. In this study, we reported the reference values of clinical chemistry and hematology parameters from healthy Chinese rhesus monkeys to benefit researchers using rhesus monkeys in experimental studies.

Materials and methods

Animals

Thirty-six healthy rhesus monkeys (*Macaca mulatta*) of either sex (18 males and 18 females) were used in the chemistry and hematology tests (19 of 3 yr, 14 of 4 yr, and 3 for 5 yr). Another 31 rhesus monkeys (16 males and 15 females) were used in thrombelastography (TEG) tests (3 to 5 yr old). The average body weight of them was 4.73 ± 0.84 kg (average body weight ± SD). These monkeys were originated from wild rhesus monkeys in South-West China, and were colony-bred in Ping'an Animal Breeding Center in Chengdu, China. The monkeys were housed in clean primate facilities and had free access to water and pelleted food. All of the monkeys bred in this center are candidates for biomedical researches. They are free of specific pathogenic microorganisms such as *Salmonella, Pathogenic dermal fungi, Shigella, Mycobacterium tuberculosis* and *Cercopithecine Heerpesvirus Type I*, according to the *National Standards of Experimental Animals in China*. The experiment procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals, West China Hospital, Sichuan University*.

Blood sample preparation

Adult rhesus monkeys were housed in dedicated primate facilities. When sample collection, the feeders slowly walked up to the animal, and gently catch it with special net. Subsequently, the head and the upper extremities of the animal were fixed by the feeder to allow collection of venous blood from the vein of lower extremity without anesthesia. Different anti-coagulant reagents were used in this study, such as 0.11 mol/l trisodium citrate for coagulation examinations, 2.0 mg/ml ethylenediamine tetraacetic acid-potassium (EDTA-K_2) for complete blood count examinations, whereas, no anti-coagulants were used in chemistry examinations. The plasma was separated by centrifugation at 2500 ***g*** for 15 min for chemistry and coagulation examinations, and the whole blood were used in routine hematology measurements. Except for TEG assay, all of the tests were performed in the Department of Experimental Medicine, West China Hospital, Sichuan University, P.R. China.

Analysis of the chemistry parameters

Chemistry measurements were carried out on sera samples, using Olympus AU5400 autoanalyzer (Tokyo, Japan). Analysis included total bilirubin (μmol/l), direct bilirubin (μmol/l), indirect bilirubin (μmol/l), total serum bile acids (TBA, μmol/l), alanine aminotransferase (ALT, IU/l), aspartate aminotransferase (AST, IU/l), alanine aminotransferase/aspartate aminotransferase ratio (AST/ALT), total protein (g/l), albumin (g/l), globulin (g/l), albumin/globulin ratio, urea nitrogen (mmol/l), creatinine (μmol/l), uric acid (μmol/l), triglyeride (mmol/l), cholesterol (mmol/l), high density lipoprotein-cholesterol (HDL-C, mmol/l), low density lipoprotein-cholesterol (LDL-C, mmol/l), alkaline phosphatase (ALP, IU/l), gamma glutamyl transferase (GGT, IU/l), creatine kinase (CK, IU/l), lactate dehydrogenase (LDH, IU/l), hydroxybutyrate dehydrogenase (HBDH, IU/l), sodium (mmol/l), potassium (mmol/l), chloride (mmol/l), calcium (mmol/l), magnesium (mmol/l), ferrum (μmol/l), inorganic phosphorus (mg/l), carbon dioxide total amount (mmol/l), and anion gap (mmol/l). Glucose (mmol/l) level was tested by glucometer with whole blood.

Analysis of the complete blood count

Complete blood count examinations were performed on the whole blood with EDTA-K_2 as anti-coagulant, using Hematology XE-2100 (Tokyo, Japan). Analysis included red blood cell count (10^{12}/l), hemoglobin (g/dl), hematocrit (%), mean corpuscular volume (fl), mean corpuscular hemoglobin (pg), mean corpuscular hemoglobin concentration (g/l), red cell distribution width coefficient of variability (%), platelet count

(10^9/l), white blood cell count (10^9/l), neutrophile count (10^9/l), lymphocyte count (10^9/l), monocyte count (10^9/l), eosinophil count (10^9/l), and basophil count (both 10^9/l).

Analysis of liver coagulation function

Coagulation function was determined on plasma samples separated from blood using trisodium citrate as anti-coagulant. Prothrombin times (PT), activated partial thromboplastin times (APTT) were determined using a STA-Stargo autoanalyzer (Paris, France). The clotting activities of factors II, VII, and X were determined using factors II-, VII-, and X-deficient human plasma, and the coagulation factor activities were calculated as PT or APTT of test plasma vs. PT or APTT of standard human plasma. Antithrombin III assays were robotically performed by the STA-Stago autoanalyzer at 405 nm using the Antithrombin III assay kit (Diagnostic STAGO, Paris, France).

TEG tests

The TEG, a measure of global hemostasis, is routinely used during cardiac and hepatic surgery to optimize blood product selection and usage [4]. In this study, the TEG of the whole blood sample has been investigated to evaluate the hemostasis function of rhesus monkey. Thrombelastograph Coagulation Analyzer (Haemoscope Corp., Chicago, IL, USA) and TEG analytical software were used in the tests. The procedure was according to the standard protocol [4].

Five main TEG parameters were measured. The reaction time (*R*) is the distance from the start of the sample run to the point of the first significant clot formation with an amplitude of 2 mm; the *K*-value is the distance (mm) taken to achieve clot strength with an amplitude of 20 mm; the alpha angle (α) reflects the kinetics of clot formation; the maximum amplitude (MA) is a measurement of maximum clot strength; the LY30 parameter measures percent lysis at 30 min after MA is reached, and the coagulation index (CI) describes the animal's overall coagulation derived from the *R*, *K*, MA and angle of native or celite-activated whole blood.

Statistic analysis

In data presentation and statistical analysis, the mean value and SD were calculated for all the determinations, and separately for the males and females. Subsequently, Independent-Samples *t*-tests were performed to identify significant differences between the values of the males and the females. An analysis of variance was performed for chemistry and hematology parameters, with sex as factor and age as covariate. Statistical significance was concluded in case of $P \leq 0.05$. SPSS 11.5 statistical software (Chicago, IL, USA) was used for data evaluation.

Results

Clinical chemistry

The baseline values of clinical chemistry parameters in 36 rhesus monkeys (18 males and 18 females) were presented in the Table 1. In terms of the significant effect of sex, the values of TBA, ALT, urea nitrogen, CK, LDH and HBDH of the male monkeys were statistically higher than that of the female monkeys. The most remarkable difference was presented in TBA (male 24.94 ± 16.74 μmol/l vs. female 12.76 ± 10.29 μmol/l, $P = 0.013$). In contrast, total protein, globulin, glucose, LDL-C, sodium, and potassium of males were significantly lower than that of the females ($P < 0.05$). A significant effect of age as covariate was calculated for globulin ($P < 0.01$), AST ($P < 0.01$), creatinine ($P < 0.05$), total protein ($P < 0.05$), and CO_2 ($P < 0.05$). The values for AST and CO_2 showed decreases with increasing ages, whereas that of globulin, creatinine, and total protein showed increases with increasing ages.

Complete blood count

The baseline values of complete blood count in 35 rhesus monkeys (18 males and 17 females) were presented in the Table 2. One sample of a female monkey was excluded for hemolysis. Statistical differences between sexes were showed in red blood cell count, hemoglobin, neutrophile count, and eosinophil count. The former two were higher in the males and the other two were higher in the females ($P < 0.05$). A significant effect of age as covariate was calculated for white blood cell ($P < 0.01$), lymphocyte ($P < 0.05$), and platelet ($P < 0.05$). The values for these parameters showed increases with increasing ages.

Liver coagulation function

Data of the baseline values of coagulation parameters in rhesus monkeys were presented in Tables 3 and 4. Plasma of 24 monkeys (12 males and 12 females) was tested with regular coagulation parameters, and TEG tests were performed with the whole blood samples of 31 monkeys (16 males

Table 1. Chemistry values of rhesus monkeys

Parameter (unit)	Males and females (n = 36)	Males (n = 18)	Females (n = 18)	Age effect
Total bilirubin (μmol/l)	2.81 ± 0.78	2.71 ± 0.78	2.83 ± 0.73	ns
Direct bilirubin (μmol/l)	0.70 ± 0.16	0.73 ± 0.19	0.68 ± 0.12	ns
Indirect bilirubin (μmol/l)	2.11 ± 0.68	2.07 ± 0.73	2.16 ± 0.64	ns
Total serum bile acids (μmol/l)	18.85 ± 15.02	24.94 ± 16.74▲	12.76 ± 10.29	ns
Alanine aminotransferase (IU/l)	53.42 ± 25.26	59.06 ± 22.36	47.78 ± 27.32	ns
Aspartate aminotransferase (IU/l)	38.67 ± 9.62	42.50 ± 9.92▲	34.83 ± 7.82	0.003↓
AST/ALT	0.83 ± 0.32	0.81 ± 0.32	0.85 ± 0.32	ns
Total protein (g/l)	78.05 ± 3.59	76.49 ± 3.30▲	79.60 ± 3.23	0.22↑
Albumin (g/l)	53.87 ± 2.67	53.43 ± 2.60	54.31 ± 2.73	ns
Globulin (g/l)	24.18 ± 2.70	23.06 ± 1.83▲	25.29 ± 3.00	0.006↑
A/G	2.25 ± 0.27	2.33 ± 0.21	2.18 ± 0.31	ns
Glucose (mmol/l)	5.72 ± 1.20	5.26 ± 1.13▲	6.19 ± 1.10	ns
Urea nitrogen (mmol/l)	7.83 ± 1.24	8.47 ± 1.21▲	7.18 ± 0.90	ns
Creatinine (μmol/l)	69.64 ± 10.24	69.73 ± 11.51	69.56 ± 9.13	0.30↑
Uric acid (μmol/l)	5.30 ± 5.33	5.81 ± 5.83	4.78 ± 4.90	ns
Triglyeride (mmol/l)	0.84 ± 0.22	0.87 ± 0.24	0.81 ± 0.20	ns
Cholesterol (mmol/l)	2.95 ± 0.52	2.85 ± 0.61	3.06 ± 0.40	ns
High density lipoprotein-cholesterol (mmol/l)	1.28 ± 0.30	1.29 ± 0.31	1.28 ± 0.31	ns
Low density lipoprotein-cholesterol (mmol/l)	1.91 ± 0.39	1.77 ± 0.42▲	2.05 ± 0.32	ns
Alkaline phosphatase (IU/l)	546.53 ± 171.43	563.61 ± 187.22	529.44 ± 157.60	ns
Gamma glutamyl transferase (IU/l)	73.67 ± 15.73	76.50 ± 16.89	70.83 ± 14.39	ns
Creatine kinase (IU/l)	207.36 ± 98.96	240.33 ± 118.75▲	174.39 ± 61.31	ns
Lactate dehydrogenase (IU/l)	513.58 ± 187.34	597.11 ± 212.44▲	430.06 ± 111.15	ns
Hydroxybutyrate dehydrogenase (IU/l)	382.31 ± 120.31	432.33 ± 139.66▲	332.28 ± 70.69	ns
Sodium (mmol/l)	151.72 ± 3.82	149.71 ± 3.07▲	153.74 ± 3.47	ns
Potassium (mmol/l)	4.78 ± 0.69	4.67 ± 0.62	4.90 ± 0.76	ns
Chloride (mmol/l)	107.14 ± 3.31	105.72 ± 3.04▲	108.57 ± 3.01	ns
Calcium (mmol/l)	2.67 ± 0.16	2.63 ± 0.13	2.72 ± 0.17	ns
Magnesium (mmol/l)	0.92 ± 0.07	0.89 ± 0.48	0.94 ± 0.9	ns
Ferrum (μmol/l)	27.98 ± 5.31	27.66 ± 4.18	28.31 ± 6.41	ns
Inorganic phosphorus (mg/l)	2.22 ± 0.50	2.14 ± 0.51	2.29 ± 0.49	ns
Carbon dioxide (mmol/l)	13.94 ± 3.77	14.90 ± 3.71	12.97 ± 3.68	0.047↓
Anion gap (mmol/l)	35.43 ± 5.68	33.76 ± 5.22	37.10 ± 1.36	ns

Data are presented as arithmetic mean values ± SD. The statistical significance is indicated with ``▲'' in the column ``males'' when comparing data of male monkeys with female monkeys (▲ means $P < 0.05$). Age effect was analyzed by sex as factor and age as covariate. P values are presented when there are statistical significances. ``↑'' means that the values showed increases with the increasing ages. ``↓'' means that the values showed decreases with the increasing ages.

Table 2. Complete blood count of rhesus monkeys

Parameter (unit)	Males and females (n = 35)	Males (n = 18)	Females (n = 17)	Age effect
Red blood cell (10^{12}/l)	5.33 ± 0.45	5.51 ± 0.40▲	5.20 ± 0.44	ns
Hemoglobin (g/dl)	127.69 ± 9.55	131.80 ± 9.29▲	124.60 ± 1.95	ns
Hematocrit (%)	0.418 ± 0.029	0.43 ± 0.02	0.41 ± 0.03	ns
Mean corpuscular volume (fl)	78.59 ± 3.59	77.65 ± 2.37	79.30 ± 4.21	ns
Mean corpuscular hemoglobin (pg)	23.98 ± 1.11	23.93 ± 0.87	24.01 ± 1.29	ns
Mean corpuscular hemoglobin concentration (g/l)	305.34 ± 8.76	308.20 ± 8.91	303.20 ± 8.23	ns
Red cell distribution width coefficient (%)	13.01 ± 0.66	13.04 ± 0.81	12.99 ± 0.54	ns
Platelet (10^9/l)	359.03 ± 71.72	353.73 ± 73.05	363.00 ± 72.35	0.012↑
White blood cell (10^9/l)	15.66 ± 3.16	14.58 ± 2.35	16.46 ± 3.48	0.026↑
Neutrophile (10^9/l)	6.18 ± 2.40	4.86 ± 1.85▲	7.17 ± 2.31	ns
Neutrophile percentage (%)	39.09 ± 11.97	33.2 ± 10.42▲	43.50 ± 11.33	ns
Lymphocyte (10^9/l)	8.94 ± 2.46	9.27 ± 2.10	8.69 ± 2.72	0.018↑
Lymphocyte percentage (%)	57.40 ± 12.61	63.67 ± 10.66	52.70 ± 12.11	ns
Monocyte (10^9/l)	0.36 ± 0.20	0.29 ± 0.19	0.40 ± 0.20	ns
Monocyte percentage (%)	2.29 ± 1.23	2.00 ± 1.31	2.50 ± 1.15	ns
Eosinophil (10^9/l)	0.07 ± 0.09	0.03 ± 0.06	0.09 ± 0.10	ns
Eosinophil percentage (%)	0.43 ± 0.61	0.20 ± 0.41▲	0.60 ± 0.68	ns
Basophil (10^9/l)	0.01 ± 0.05	0.00 ± 0.00	0.02 ± 0.06	ns
Basophil percentage (%)	0.06 ± 0.24	0.00 ± 0.00	0.10 ± 0.31	ns

For explanation, see legend to Table 1.

Table 3. Coagulation function of rhesus monkeys

Parameter (unit)	Males and females (n = 24)	Males (n = 12)	Females (n = 12)
Prothrombin times (s)	14.18 ± 0.96	14.49 ± 0.86	13.86 ± 0.99
Activated partial thromboplastin time (s)	43.04 ± 5.43	41.80 ± 6.15	44.28 ± 4.52
Factor II	107.63 ± 13.53	105.58 ± 14.68	109.67 ± 12.56
Factor VII	299.33 ± 3.06	298.75 ± 4.33	299.91 ± 0.29
Factor X	84.50 ± 7.68	87.17 ± 7.67	81.83 ± 6.99
Antothrombin-III	86.17 ± 13.87	84.17 ± 12.71	88.17 ± 15.22

For explanation, see legend to Table 1.

Table 4. Thromboelastograph of rhesus monkeys

Parameter (unit)	Males and females (n = 31)	Males (n = 16)	Females (n = 15)
R (min)	4.62 ± 1.00	4.68 ± 0.99	4.54 ± 1.03
K (min)	1.23 ± 0.30	1.16 ± 0.26	1.31 ± 0.32
Angel (deg)	72.92 ± 3.86	73.85 ± 3.32	71.93 ± 4.25
MA (mm)	75.17 ± 6.72	76.66 ± 6.59	73.58 ± 6.69
LY30 (%)	1.33 ± 1.37	0.77 ± 0.94▲	1.93 ± 1.52
CI	3.47 ± 1.33	3.70 ± 1.39	3.22 ± 1.27

For explanation, see legend to Table 1.

and 15 females). The result of PT, APTT, and the activities of coagulation factors showed no significant difference between male and female rhesus monkeys. Furthermore, the results of TEG tests showed no statistical difference between the males and the females, except for LY30 which was 2.5-fold higher in the females (male 0.77 ± 0.94% vs. female 1.93 ± 1.52%).

Discussion

We reported here the baseline values of clinical chemistry, hematology, and coagulation parameters of Chinese rhesus monkeys. These data are valuable references to evaluate the functions of liver, kidney, pancreas, and heart in experimental monkeys, and will benefit to identify the pathological conditions after special treatments in the monkeys.

Baboon and cynomolygus monkey are another two species of widely used Old World Monkeys in biologic researches, and cynomolgus monkey and rhesus monkey are both belong to macaques. Previously, Schuurman reported the reference values of clinical chemistry and clinical hematology parameters in baboon and cynomolgus monkey from USA [5,6]. Comparisons of the results suggest highly similarities in clinical chemistry and hematology parameters in these three species of non-human primates. However, some obvious differences are found in the following important parameters. In terms of chemistry parameters, the total bilirubin of cynomolgus is 2.78- and 1.99-fold higher than that of rhesus and baboon, respectively; the ALT of cynomolgus is similar to that of rhesus, but is 1.57-fold higher than that of baboon; the total protein and globulin levels of cynomolgus are both obviously higher than rhesus and baboon; the creatinine of cynomolgus and baboon are 1.43- and 1.19-fold higher than rhsus, respectively; and the LDH of rhesus is 1.5- and 1.78-fold higher than cynomolgus and baboon, respectively. In terms of hematology parameters, the white blood cell count, neutrophile and lymphocyte count of rhesus are similar to that of cynomolgus but are 1.98-, 1.87- and 2.08-fold higher than that of baboon, respectively. Therefore, it is relevant to consider the species in proper evaluation of experimental data. Moreover, a lot of chemistry and hematology parameters, particular coagulation parameters involved in our research are not reported in baboon and cycomolgus, also other non-human primates. Our results will thus provide valuable supplements to our knowledge of non-human primates.

Furthermore, when interpreting our results of rhesus monkeys, what should be noted is that the blood samples were collected without anesthesia. So, the monkey was under stress for some extent, which possibly interfered the final results of some parameters sensitive to stress. Moreover, subclinical disease (particularly parasitic) might have contributed to the high serum liver activities. For example, parasitic infection such as blood fluke, ameba, and plasmodium may result in high serum liver enzyme activities but low white blood cell count. On the other hand, virus infection may not only increase the lymphocyte level, but also affect the liver function. In our research, these experimental animals are through routine health screening and are confirmed to be free of five regular infectious pathogens, such as *Salmonella, Pathogenic dermal fungi, Shigella, Mycobacterium tuberculosis* and *Cercopithecine Heerpesvirus Type I*. However, comprehensive screening of other major pathogens has not been performed, such as Herpes B, simian T-cell leukemia virus, simian retrovirus D, plasmodium as so on, which should be considered when interpreting the data from experimental monkeys.

Regarding the effect of age, it is a relative small range of age in these monkeys. Particularly, besides of the most monkeys of 3 and 4 yr, only three monkeys of 5 yr involved. So, a larger sample size is needed to confirm the significant effect of age.

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