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Clinical

Hematological values of mice and rat strains : Perspectives from ACTREC Laboratory Animal Facility

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Abstract

Experimental results of the hematological or biochemical data are best interpreted and compared based on previously reported baseline data for the strains in question. Maintenance and managemental conditions of the animals varies from organization to organization. Naturally, the baseline data for hematology and biochemistry may vary amongst the same species or strains of animals. This study was undertaken to establish hematological reference values in mice and rat strains maintained at Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai and to compare with that of the values reported by other workers as in the literature. Hematological values were found to be within the normal range when compared to the values reported in the literature. Slight deviations in these values have been noticed within the strain. However, they remain within the reported normal range. Most of the time, the normal range values were so wide that it necessitates the need for the laboratory-specific baseline data. The wide range may be the result of genetic changes, change in feed, housing conditions, technique and equipments used or management conditions. The hematological values of these strains may provide valuable baseline data to the researchers for future experiments using these inbred strains.

Key words: laboratory animals, hematology, rats, mice

Introduction

Rodents are the most frequently used animals worldwide in biomedical research. Use of inbred mice and rats in cancer and biomedical research has made possible many contributions of fundamental and productive nature that would not have been possible otherwise. Although several studies were conducted using different strains of mice and rats, variations in biological characteristics were extremely common between inbred strains of mice and rat (Festing, 1979). Studies on rodents provide clinical toxicity predictions that may be comparable to studies in other species (Harrison *et al.* 1978).

Number of mice and rat strains in use for various studies differs at different organizations. Information concerning the origin, behavior, physiology, anatomy, reproduction, disease incidence, hematology and biochemical values of almost all the strains has been well documented. The values reported so far in the literature serve as standard baseline data in research. However, many a times reported values in the literature do not exactly match during the experimentation in different laboratories. Slight variation in hematological indices may be seen in mice of the same strain from different laboratories, but significant difference may also be seen between mice of different strains. This indicates that, the normal values may differ from laboratory to laboratory due to either genetic change, change in environment or feed (Green, 1966). However, the values obtained in one strain are always under those specific conditions and should not be construed to reflect data for that strain in all circumstances. Hence, it was felt necessary to determine the reference values for hematological parameters under our housing conditions for mice and rats strains at ACTREC, Navi Mumbai. The present study deals with the hematological reference values that

includes hemoglobin (Hb), red blood cells (RBC) count, white blood cells (WBC) count, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and differential leucocytes count (DLC).

Materials and Methods

The mice and rat strains maintained at ACTREC Animal Facility and used in the present study are listed in Table 1 and 2. Breeding nuclei of these strains have been procured in the past either from the Jackson Laboratories, USA, or Charles River Laboratories, USA and then inbred at ACTREC. For determination of hematological parameters, 6-8 wks old specific pathogen free animals of each sex (n=5) were randomly selected. Five animals were housed in each cage under normal animal housing conditions of 23 ± 2 ^{o}C temperature, 55 ± 5 % relative humidity and 12 h darkness and 12 h illumination. The animals were fed on pelleted feed prepared in-house at the facility as per the nutritional requirements (CPCSEA guidelines, March 2008) and were given UV treated drinking water *ad libitum*. The animals were free for antibodies of CAR bacillus, *Clostridium piliformae* (CP), Ectromelia virus Hantaan virus, Kilham rat virus, Lymphocytic Choriomeningitis virus (LCMV), *Mycoplasma pulmonis* (MP), Pneumonia virus of mice (PVM), Reo3 virus and Sendai virus as screened by ELISA based methods using commercially available kits from XpressBio Life Sciences Products. The Institutional Animal Ethics Committee of the ACTREC has approved the use of animals.Two hundred micro liters of blood was collected directly from orbital plexus of the mice without anesthesia whereas in rats, under mild anesthesia by Ketamin injection at the dose rate of 10 mg/kg intramuscularly. Blood smears were prepared and stained with Leishman's stain for Differential Leucocytes Count (DLC).

Hematological parameters like RBC and WBC were carried out using automated counter (Sysmex, model no. KX-21, Japan). For RBC's, three parameters viz total RBC count, Hb concentration and PCV percentage were measured whereas for WBC's, total WBC count and their proportions were measured by automated counter.

Results

Hematological parameters of mice and rat strains determined in this study are presented in Table 1 and 2 respectively. Average Hb values of all the mice and rat strains maintained at ACTREC were found to be 14.5 and 15.6 gm/dl respectively. The average RBC values of mice and rat strains were found to be 8.6 and 8.8 millionos/cu.mm and average PCV values were found to be 45.8 and 49 % respectively.

Table 1. Hematological data in mice strains

No	Strain	Hb gm/dl	RBC 10^6/cu mm	PCV %	MCV cu μ	MCH μμgm	MCHC gm/dl	TLC /cu mm	Differential Leucocytes Count (DLC) %			
									N	E	L	M
1	BALB/c	14.9 ± 0.4	8.9 ± 0.3	46 ± 2.6	52 ± 2.8	17 ± 0.6	33 ± 1.4	6680 ± 964.7	24 ± 8.9	1 ± 0.5	74 ± 8.8	1 ± 0.8
2	C3H	14.5 ± 0.5	7.9 ± 0.7	45 ± 3.0	49 ± 3.4	17 ± 0.5	32 ± 1.3	7200 ± 1500	41 ± 5.3	1 ± 0.9	57 ± 4.8	1 ± 0.6
3	C57BL/6	14.6 ± 0.4	8.8 ± 0.2	47 ± 2.0	53 ± 2.0	17 ± 0.5	31 ± 1.3	7418 ± 2222	18 ± 5.8	1 ± 1.1	80 ± 6.0	1 ± 0.7
4	CD-1	13.2 ± 0.9	7.8 ± 0.5	42 ± 2.7	54 ± 2.3	17 ± 0.6	31 ± 1.0	7767 ± 2790	18 ± 5.2	3 ± 1.7	78 ± 5.4	1 ± 0.7
5	Cr:ORL SENCAR	14.8 ± 0.3	9.0 ± 0.2	49 ± 2.0	55 ± 2.4	17 ± 0.5	30 ± 1.0	6668 ± 1109	23 ± 8.5	1 ± 1.0	75 ± 8.3	1 ± 0.5
6	DBA/2	14.5 ± 0.4	8.8 ± 0.2	45 ± 2.0	51 ± 1.7	17 ± 0.5	32 ± 1.2	7510 ± 747.5	17 ± 4.0	1 ± 0.7	81 ± 6.7	1 ± 0.5
7	ICRC	14.5 ± 0.7	8.7 ± 0.4	45 ± 2.7	51 ± 2.0	17 ± 0.4	32 ± 1.7	6650 ± 1844	24 ± 5.7	1 ± 0.7	74 ± 5.7	1 ± 0.6
8	S/RV/Cri	14.7 ± 1.5	8.6 ± 0.8	46 ± 4.4	53 ± 1.2	17 ± 0.6	31 ± 0.6	6950 ± 1260	26 ± 12.3	2 ± 0.7	71 ± 1.4	1 ± 0.7
9	S/RV/Cri-ba	14.6 ± 0.5	8.8 ± 0.3	47 ± 3.5	53 ± 2.6	17 ± 0.4	31 ± 1.4	6150 ± 2063	57 ± 9.7	1 ± 0.7	40 ± 9.3	2 ± 0.8
	Mean	14.5 ± 0.5	8.6 ± 0.4	45.8 ± 1.9	52 ± 1.8	17 ± 0	31 ± 0.9	6999 ± 515.2	28 ± 13.2	1 ± 0.7	70 ± 13.3	1 ± 0.3

N- Neutrophils, E- Eosinophils, L- Lymphocytes, M- Monocytes

Table 2. Hematological data in rat strains

No	Strain	Hb gm/ dl	RBC 10^6/cu mm	PCV%	MCV cu μ	MCH μμgm	MCHC gm/dl	T.L.C. /μl	Differential Leucocytes Count (DLC) %			
									N	E	L	M
1	S.D.	15.6 ± 0.7	9.0 ± 0.4	49 ± 2.3	54 ± 1.2	17 ± 0.0	32 ± 0.6	13800 ± 2130	16 ± 3.9	1 ± 1.6	82 ± 3.2	1 ± 0.6
2	Wistar	15.8 ± 0.3	8.0 ± 0.2	51 ± 1.9	55 ± 1.9	17 ± 0.4	31 ± 0.9	12125 ± 2733	18 ± 5.7	1 ± 0.9	79 ± 6.0	2 ± 0.5
3	F344	15.4 ± 0.3	9.3 ± 0.3	46 ± 2.1	49 ± 1.4	16 ± 0.5	33 ± 1.3	10363 ± 1069.0	14 ± 3.3	1 ± 0.5	84 ± 3.7	1 ± 0.5
	Mean	15.6 ± 0.2	8.8 ± 0.7	49 ± 2.5	53 ± 3.2	17 ± 0.6	32 ± 1.0	12096 ± 1718.7	16 ± 2.0	1 ± 0	82 ± 2.5	1 ± 0.6

N- Neutrophils, E- Eosinophils, L- Lymphocytes, M- Monocytes.

Average MCV values of mice and rat strains were found to be 52 and 53 cubic microns and average MCH values were found to be 17 and 17 mmgm respectively. It was observed that average MCHC values in mice and rat strains were 31- 32 gm/ dl respectively. Average TLC values of mice and rat strains were found to be 6999 and 12096 per cubic mm respectively

The differential leucocyte counts were determined in rats and mice and presented in Table 1 and 2. Average neutrophil counts in mice and rat strains were found to be 28 and 16 %, eosinophils were 1 and 1 %, lymphocytes were 70 and 82 % and monocytes were found to be 1 and 1 % respectively.

Discussion

Hematological values are reported to be influenced by variety of parameter which includes site of sample collection, sex, strain, age, stress level of animals, method of restraint, and type of anesthesia (Thrall, 2004). Route of blood withdrawal as well as analytical method and equipment used may result in different values for some of the parameters (Lang, 1993).

Most of the hematological investigations include total count and proportional values of RBC and WBC's. Type of food and change in managemental practices are reported to influence the change in hematological parameters in the same strain of rodents. For this reason, the present study was designed to study hematological values of rodent strains housed at ACTREC under standard housing conditions. Values obtained in the present study were compared with the reported literature. Hematological values can also help to detect the population drift or analytical problems within a laboratory (Giknis, 2008).

Hemoglobin amongst all strains of mice showed similar values as reported by Aiso *et al.* (2005), Alter *et al.* (1974), Charles River Laboratories (CRL) Technical Bulletin (1986), Hariharan (1979), Harrison *et al.* (1978) and Hejtmancik *et al.* (2002). However, Kawabe *et al.* (1993) reported that 17 % of hemoglobin as normal value in one of their experiments on B_6C_3F1 mice whereas Merchant and Modi (2004) has reported 8.9-10.6 gm % of Hb as normal values for mice in their experiments. The CRL Technical bulletin (1986) has even reported 8.2- 16.2 gm % Hb values in CD1 mice as baseline values. These reports indicate that the range of Hb values in case of mice strains may vary from 8.2-17 gm %. Lower values shown here may be normal for one laboratory but may be lower for another laboratory where normal values for the mice strains are 17 gm % and vice versa..

Hemoglobin values in rats were found to be similar to that reported by various investigators. Hemoglobin values reported were 16.1 gm% (Aiso *et al.* 2005); 13.6% (Alter *et al.* 1974); 11-17gm% (Bhardwaj, 1992); 12.3 gm% (Bhilegaonkar *et al.* 1995); 14.1-18.2 gm/dl (Charles River Lab. Tech. Bulletin, 1982; 1984); 13.7- 17.6 gm% (Giknis *et al.* 2008), 12.0-17.5 gm% (Hariharan ,1980); 14.0- 16.5 gm% (Hejtmancik *et al.* 2002); 9.19- 16.82 gm% (Lang, 1993), 13.6- 14.9 gm% (Lovell *et al.* 1981); 14.8-15.8 gm% (Saibaba *et al.* 1995); 14.72 gm/dl (Stana *et al.* 2009); 14.3- 15.3 gm% (Walter , 1999) and 15.7 gm% (Waynforth, 1980). On the contrary Jonkar and Till (1995) has reported 9.6 gm/ dl and Weber *et al.* (2002) has reported 8.2- 14 gm/dl of Hb as normal values in their experiment using rats. This value (8.2 mg/dl) is significantly low as compared to hemoglobin values reported in the literature by others. This suggests that there are differences in baseline values of Hb among different laboratories and ranges from 8.2- 18.2 gm%.

Total Red Blood Cells (RBC) values in all strains of mice at ACTREC were found to be varying between 8- 9 10^6/cu mm, which are comparable with the value reported by Aiso *et al.* (2005); Charles River Lab. Tech. Bulletin (1986); Hariharan (1979); Hejtmancik *et al.* (2002); Kawabe et. al. (1993) and Merchant *et al.* (2004). Alter *et al.* (1974) has reported 6.89 10^6/cu mm as RBC values in B6D2F1 mice which is significantly low when compared to the values reported in the literature. The review of the data indicates that the normal RBC values in mice strains may vary from 6.89- 9 10^6/cu mm.

The RBC values in all rat strains at ACTREC were found to be in the range of 8.0- 9.3 10^6/cu mm that are similar to the values reported by Aiso *et al.* (2005); Bhardwaj (1992); Giknis (2008); Hejtmancik *et al.*(2002); Jonkar and Till (1995) and Waynforth (1980). However, Saibaba *et al.* (1995) has reported 6.9 10^6/cu mm of total RBC values as normal value in Wistar rats. Lovell *et al.* (1981) and Walter *et al.* (1999) has reported the RBC values ranging from 6.18- 8.47 10^6/cu mm. Weber *et al.* (2002) has even reported 6.92- 11.21 10^6/cu mm as normal values for RBC's in case of Wood rats. Normal range of RBC values in case of rat strains are seen varying from 6.1- 11.21 10^6/cu mm.

Total Leucocyte Counts in all the strains of mice were found to be in the range 6.2- 7.8 thousands/cu mm which is comparable to the values reported by Charles River Lab. Tech. Bulletin (1986); Hariharan (1979); Harrison (1978), Kawabe *et al.* (1993) and Merchant and Modi (2004).

Total Leucocyte Counts in CRI rat strains was found to be in the range of 10363- 13800/cu mm which is comparable to the values reported by Bhardwaj (1992); Charles River Laboratories Technical Bulletin (1982) and Hariharan (1980). However, Alter *et al.* (1974) and Saibaba *et al.* (1995) have reported 5000 and 5360/cu mm for Wistar and SD rats, respectively as normal values. Even Lovell *et al.* (1981) has also reported the TLC values ranging from 3.7- 7.6 thousands/ cu mm. This indicates that normal TLC values may ranges from 3.7-13.8 thousand/ cu mm in rats.

Packed Cell Volume values in mice strains were found to be varying between 42- 47 %. Hariharan (1979); Harrison *et al.* (1978); Hejtmancik *et al.* (2002) as well as Kawabe et. al. (1993) has reported similar values as normal in their experiment. Aiso *et al.* (2005) has reported 50.2 % as PCV values in BDF1 mice. The PCV values in mice ranges from 42-50% as reported in the literature.

Packed Cell Volume values in rats strains maintained in ACTREC were found to be varying in the range of 46- 51 % which are comparable to the values reported by Aiso *et al.* (2005); Giknis (2008); Hariharan (1980); Hejtmancik *et al.* (2002); Lang (1993); NLAC News Letter (1992) and Waynforth (1980). Weber *et al.* (2002) has reported PCV values ranging from 26.9- 48.9 % whereas Alter *et al.* (1974) has reported PCV values as 38.4 %. The PCV values in rats varying in the range of 26.9- 51 % as reported in the literature.

Average Mean Corpuscular Volume (MCV) values in all mice and rat strains were found to be varying in the range of 49- 55 cubic micron which matches with the values reported by Aiso *et al.* (2005); Charles River Lab. Tech. Bulletin (1982); Giknis (2008); Hariharan (1979); Hejtmancik *et al.* (2002); Jonkar and Till (1995); Lang (1993); Merchant and Modi (2004) and Walter (1999). Lovell *et al.* (1981) has reported MCV values towards higher side in the range of 58- 67 cubic micron.

Mean Corpuscular Hemoglobin (MCH) values in all mice and rat strains were found to be varying in the range of 16- 17 mmg which are comparable to the values reported by Aiso *et al.* (2005); Alter *et al.* (1974); Charles River Lab. Tech. Bulletin (1982); Giknis (2008) and Weber *et al.* (2002). Walter (1999) and Lovell *et al.* (1981) has reported the MCH values in the range of 18.0- 22.0 mmg which are towards higher side as compared to all other references reported above. On the contrary, Merchant and Modi (2004) have reported only 11.3- 12.5 mmg of MCH values in mice. The review of this index shows that the MCH values in mice and rats may vary from 11.3- 22.0 mmg.

Mean Corpuscular Hemoglobin Concentration (MCHC) values in mice strains were found to be varying in the range of 30- 33 gm/dl which are comparable to the values reported by the Charles River Lab. Tech. Bulletin (1986) where normal range for their mice are shown varying from 31 to 39 gm/dl. Hejtmancik *et al.* (2002) has reported 32- 33 gm/dl MCHC values in case of B6C3F1 mice. These results and literature survey indicates that the control values of MCHC in case of mice may vary from 30- 39 gm/dl.

Mean Corpuscular Hemoglobin Concentration (MCHC) values in rat strains were found to be varying in the range of 31- 33 gm/dl which are comparable to the values reported by the Charles River Laboratories Technical Bulletin (1984); Lovell *et al.* (1981); Walter (1999) and Weber *et al.* (2002). Alter *et al.* (1974) has reported 35 gm/dl of MCHC values for their rats. Hejtmancik *et al.* ((2002) has reported 33.5- 35.2 gm/dl of MCHC values in case of F344 rats whereas Giknis (2008) has reported MCHC values of CRL:WI(Han) rats in the range of 32.9- 37.5 gm/dl. From the above literature, it is observed that MCHC values are in the range of 31- 37.5 gm/dl.

Differential Leukocytes Counts determined in all mice and rat strains were comparable to earlier reports by Bhardwaj (1992), Charles River Laboratories Technical Bulletin (1982); Charles River Laboratories Technical bulletin (1986); Giknis (2008); Hariharan (1979) and Lovell *et al.* (1981) except for the strin S/RV/Cri-ba which shows reversal in values in case of lymphocytes and neutrophils. The S/RV/Cri-ba is a hairless mutant strain of mice originated from the Swiss strain of mice at the Cancer Research Institute, Mumbai (Randelia and Sanghvi, 1961). The reversal in lymphocytes and neutrophils values of DLC in this strain need to be further examined and characterized.

It is evident that hematological values of mice and rat strains in ACTREC are comparable to the values reported in the literature. Male and female animals did not show significant differences in the hematological parameter studied. However, in some cases the range of reported normal values in the literature from laboratory to laboratory are so wide and this could be due to some of the factors like either feed, managemental practices, route of blood withdraw or equipments used for testing. Only slight deviation in these values has been noticed amongst the strains maintained in ACTREC except the strain S/RV/Cri-ba where the neutrophils and lymphocyte values are seen reversed. The varying levels of normal values of the hematological parameters from laboratory to laboratory highlight the importance of determination of normal base line hematological and biochemical data for their own animal strains.

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