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Article

Performance of complete blood count (CBC) upon use of different anticoagulants in rats

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Abstract: Complete blood count (CBC) is used as an index of health status of human and different animals as well as to diagnose a variety of diseases. Therefore, there is a growing need of using the most suitable anticoagulant to obtain the most appropriate hemogram. The present study was designed to assess the effect of different anticoagulants viz. Heparin, Sodium Citrate and EDTA on complete blood count (CBC) in rat with a view to choosing the best suitable candidate among the common anticoagulants. A total of 30 samples out of which 10 were for each type anticoagulant were collected from 10 apparently healthy rats of Long Evans strain. From each rat 6 ml of blood was drawn and subsequently divided into three different test tubes with three different anticoagulants. The samples were analyzed for their complete blood count (TEC, TLC, Hb, Hct, DLC, absolute leukocyte count, Red Cell Indices, RDW-SD, RDWCV, Platelet, MPV, PCT and PDW) using Sysmex XT-1800i Auto hematological analyzer. Results showed a significant ($p<0.05$) decrease in TLC, absolute neutrophil, and platelet count, and a significant ($p<0.05$) higher level of relative lymphocyte count in Sodium Citrate treated sample than with Heparin and EDTA. No significant changes were observed for RBC or erythrocyte indices among the three different groups. It can be concluded that samples treated with Sodium Citrate results in significant change in blood parameters. Therefore, it is better to use Heparin and EDTA than Sodium Citrate for complete blood count (CBC).

Keywords: complete blood count (CBC); heparin; sodium citrate; EDTA; rat

1. Introduction

Blood is a connective tissue made up of cells suspended in a fluid medium known as plasma which is made up of minerals, salt, vitamins, coagulation factors and organic elements. The cellular portion of blood is composed of Erythrocytes (RBC), Leukocytes (WBC) and Platelets (Wood *et al*., 1999). In present days Complete blood count (CBC) is one of the most choices of test for blood that is frequently used in veterinary medical profession. CBC consists of investigation of cellular content of blood mainly white blood cell, thrombocytes and the red blood cell with related parameters (packed cell volume, hemoglobin concentration) (Wood *et al*., 1999). To carry out this operation the whole blood needs to be collected in a suitable anticoagulant to prevent undesirable clotting. Several anticoagulants e.g. EDTA (Ethylene Diamine Tetraacetic acid), heparin, sodium citrate, oxalate, ACD (Acid Citrate Dextrose), CPD (Citrate Phosphate Dextrose) are used for collection and routine laboratory analysis. These anticoagulants have effects on hematological parameters. Heparin is a naturally synthesized endogenous anticoagulant which prevents blood coagulation in vivo as well as in vitro. It inhibits

the conversion of prothrombin into active thrombin, and thus prevents conversion of fibrinogen into fibrin (Witeska and Wargocka, 2011).The mechanism of action of EDTA and sodium citrate is based on inhibition of thrombocyte aggregation and various reactions of hemostatic cascade due to chelation of free Ca^{2+} ions (Witeska and Wargocka, 2011). However, there is very little evidence of the effects of anticoagulants on blood parameters of various animals. In human hematology, it is assumed that heparin should not be used for blood smears due to blue staining background, or for WBC count due to clotting leukocytes, while EDTA is inappropriate for evaluation of erythrocyte osmotic fragility, and its excess may cause damage to the blood cells (Marianska *et al.*, 2003). In mammals, K_2EDTA is the recommended anticoagulant for storing blood samples for hematologic testing. In many domestic species, heparin alters leukocyte morphology, provides poorer staining quality to the cells, and does not prevent platelet aggregation (Knoll, 2000). Most recently; a study in macaws compared the temporal effects of 3 commonly used anticoagulants. However the use of heparin coated syringes in this study limited the ability to fully ascertain the effect of individual anticoagulants (Harr *et al*., 2005). Citrate and EDTA are recommended for samples which are analyzed by automated methods. Heparin has been advocated for use with blood samples from species with nucleated RBCs based on the perceived observation of hemolysis in EDTA-preserved samples in some reptiles and birds. The effects of various types of anticoagulants in hematology were studied in various animal, bird and fish species in a scattered way by few researchers and still the thorough investigation in this area is of greater importance. Therefore, the present study was undertaken to determine the effects of three commonly used anticoagulants Heparin, Sodium Citrate and EDTA on complete blood count (CBC) of rats.

2. Materials and Methods

The study was conducted in the Department of Physiology, Faculty of Veterinary science, Bangladesh Agricultural University, Mymensingh.

2.1. Experimental animals

A total of 15 rats of Long Evans strain aged between 18-20 days were purchased from International Center for Diarrheal Disease Research, Bangladesh (icddr'b), Mohakhali, Dhaka and reared in the compartmentalized square wooden cages wrapped with wire mesh under controlled conditions of temperature (26-30)°C and relative humidity of 70-80% with natural day light.

2.2. Preparation of the experimental laboratory

The laboratory was cleaned and washed with disinfectant. Then it was left empty for 3 days before placing the experimental rats. All necessary equipments were set properly for proper handling and care of the animals.

2.3. Experimental design

After acclimatization, all (15) rats were reared in same environmental condition and out of 15 rats 10 were randomly chosen at the age between 45-48 days. Each rat is designated as 1, 2, 3, and so on. For each rat 3 tubes were used to collect blood sample which were marked as 1.a, 1.b, 1.c for rat no.1, 2.a, 2.b, 2.c for rat no.2 and so on where 'a' for EDTA, 'b' for Heparin and 'c' for Citrate.

2.4. Management practices

Commercial rat pellet was collected from icddr'b and was supplied to all rats throughout the experiment. The feed was supplied twice daily and fresh potable drinking water was available. Proper hygienic and sanitary measurement was taken during the experimental period.

2.5. Blood sample collection

Before sample collection the rats were kept fasting overnight. Blood samples were collected from rats under general anesthesia by using diethyl ether. A total of 30 samples out of which 10 were for each type anticoagulant were collected from 10 apparently healthy rats. From each rat 6 ml of blood was drawn and subsequently divided into three different test tubes commercially treated with three different anticoagulants. K_3EDTA (3.6 mg/2 ml), Lithium Heparin and Sodium citrate (3.2%) containing commercially available blood

collection tubes were used for sampling. Under general anesthesia the abdomen of the animal was opened and blood was drawn from the bifurcation of the abdominal aorta. Prompt transfer of the blood into the tube and proper mixing with the anticoagulant was ensured immediate after collection. Then each and every samples were sent to laboratory for the measurement of hematological parameters viz; RBC, WBC, platelet count, DLC, Hb level, hematocrit value and measurement of MCV, MCH, MCHC. All samples were analyzed by Sysmex XT- 1800i Automated Hematology Analyzer.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine the effect of different anticoagulant on blood parameters. Bonferroni's multiple comparison tests was applied for post-hoc comparison. All statistical analyses were performed using a commercial statistical program- SPSS.

3. Results and Discussion

3.1. Effects on total erythrocyte count and erythrocyte indices

The results placed in Table 1 show no significant differences in total RBC count and Hb level among three anticoagulant samples. These findings were partially similar with Mafuvadze *et al*. (2007) findings but differ from Faggio *et al.* (2014) who reported a higher level of Hb in EDTA sample as compared to heparin and citrate sample. In contrast, it also differs from the findings of Muro *et al.* (1998) who reported a lower level of RBC in EDTA sample. The hematocrit (Hct) value of sodium citrate is 35.95 ± 1.48 and is significantly ($P<0.01$) lower than that of the heparinized sample (41.41 ± 3.02) and is closely agreeable with the findings of Harr *et al.* (2005). The MCV showed significant ($P<0.01$) lower level of its value in the sample with sodium citrate compared to EDTA and heparinized sample that is related with findings of Uko *et al.* (1996) and Maqbool *et al*. (2014). The MCHC is also significantly ($P< 0.05$) lower in sodium citrate than that of EDTA and heparin sample. The values of MCH and red cell distribution width (RDW %) also shows no significant variation among the samples preserved in different anticoagulants.

3.2. Effects on total leukocyte count and absolute count of its cell component

The absolute count of total leukocyte in citrated blood sample (5.81 ± 1.56) is significantly ($P<0.01$) lower than that in the EDTA (10.31 ± 2.03) and heparinized sample (8.99 ± 2.65) which agrees the findings of Faggio *et al*. (2004). The result represented a significant decrease in the count of neutrophil ($P<0.01$), lymphocyte ($P<0.05$), monocyte ($P<0.01$) and eosinophil ($P<0.01$) in citrated blood sample compared to samples with EDTA and heparin as shown in Table 2. This finding supports the previous report of Adeyemo *et al.* (2009); Faggio *et al*. (2004) and Hanley *et al.* (2004) except the value of monocyte count. Neither of the anticoagulants showed significant effect on absolute count of basophil.

3.3. Effects on differential leukocyte count

The values of differential leukocyte count are placed in Table 3 showing a significant ($P<0.01$) decrease in relative count of neutrophil, monocyte and eosinophil in case of sample preserved in sodium citrate but a significant ($P<0.05$) increase in relative lymphocyte count that is similar with the findings of Guzman *et al.* (2008). It revealed that the difference in anticoagulant for preservation of blood sample does not matter in relative count of basophil.

3.4. Effects on platelet count and its indices

Effects on Platelet count and its indices are placed in the Table 4. The value of MPV (fL) showed a significant ($P<0.05$) level of decrease in citrated sample (1.12±2.96) compared to EDTA (5.77± 3.95$^{)}$ and heparinized (6.44 ± 2.90) sample. The findings of this study showed that difference in choosing different anticoagulant do not affect platelet count and PCT (%) measurement. These findings were disagree with McShine *et al.* (1990) who reported a lower platelet count in citrated sample than those in EDTA sample.

Table 1. Effects of K_3EDTA, heparin and sodium citrate on total erythrocyte count and erythrocyte indices.

Parameters	Mean ± SD (Range)			Level of significance	P value
	K_3EDTA	Heparin	Sodium citrate		
RBC (M/µL)	6.11 ± 0.58 (4.89-6.66)	6.62 ± 0.65 (5.42-7.25)	6.16 ± 0.28 (5.75-6.50)	NS	0.172
Hb (g/dL)	13.43 ± 0.71 (12.34-14.50)	14.05 ± 1.26 (12.00-15.30)	12.96 ± 0.49 (12.00-13.40)	NS	0.095
Hct (%)	39.85 ± 3.82^{ab} (32.30-43.10)	41.41 ± 3.02^{a} (37.00-45.10)	35.95 ± 1.48^{b} (32.90-37.20)	**	0.008
MCV (fL)	65.27 ± 1.91^{a} (63.02-68.72)	62.80 ± 4.09^{a} (57.22-68.32)	58.34 ± 2.06^{b} (55.22-61.92)	**	0.001
MCH (pg)	22.12 ± 1.44 (21.22-25.24)	21.24 ± 0.74 (20.02-22.12)	21.05 ± 0.47 (20.42-21.82)	NS	0.121
MCHC (g/dL)	33.76 ± 2.10^{a} (31.40-38.14)	33.91 ± 1.22^{a} (32.40-35.40)	36.04 ± 0.65^{b} (35.20-37.04)	*	0.04
RDW-CV (%)	14.6 ± 0.68 (13.70-15.60)	15.14 ± 1.04 (13.90-16.71)	15.28 ± 0.73 (14.30-16.31)	NS	0.290

**= Significant at P<0.01; *= Significant at P<0.05; NS= Non significant

Table 2. Effects of K_3EDTA, heparin and sodium citrate on total leukocyte count and absolute count of its cells component.

Parameters	Mean ± SD (Range)			Level of significance	P value
	K_3EDTA	Heparin	Sodium citrate		
WBC (K/µL)	10.31 ± 2.03^{a} (8.29-13.57)	8.99 ± 2.65^{a} (5.10-13.0)	5.81 ± 1.56^{b} (3.67-8.11)	**	0.003
Neutrophil (K/µL)	2.20 ± 0.37^{a} (1.60-2.66)	1.72 ± 1.33^{a} (0.28-4.32)	0.36 ± 0.32^{b} (0.05-0.81)	**	0.002
Lymphocyte (K/µL)	7.59 ± 1.84^{a} (5.45-11.01)	6.96 ± 1.37^{ab} (4.74-8.22)	5.36± 1.28^{b} (3.26-7.15)	*	0.041
Monocyte (K/µL)	0.20 ± 0.05^{a} (0.13-0.32)	0.17 ± 0.12^{a} (0.03-0.37)	0.03 ± 0.03^{b} (0.01-0.09)	**	0.013
Eosinophil (K/µL)	0.30 ± 0.26^{a} (0.05-0.84)	0.10 ± 0.04^{ab} (0.04-0.15)	0.03 ± 0.02^{b} (0.00-0.05)	**	0.011
Basophils (K/µL)	0.014± 0.005 (0.01-0.02)	0.014 ± 0.007 (0.00-0.02)	0.007 ± 0.004 (0.00-0.01)	NS	0.068

**= Significant at P<0.01; *= Significant at P<0.05; NS= Non significant

Table 3. Effects of K_3EDTA, heparin and sodium citrate on differential leukocyte count.

Parameters	Mean ± SD (Range)			Level of significance	P value
	K_3EDTA	Heparin	Sodium citrate		
Neutrophil (%)	21.94 ± 5.13^{a} (15.54-31.24)	17.15 ± 9.02^{a} (5.54-33.24)	5.84 ± 4.29^{b} (1.02-10.74)	**	0.001
Lymphocyte (%)	73.14 ± 5.10^{a} (65.74-81.14)	79.14 ± 9.72^{a} (63.24-92.94)	92.93± 4.94^{b} (87.24-98.78)	**	0.007
Monocyte (%)	1.96 ± 0.51^{a} (1.34-2.94)	1.82 ± 0.94^{a} (0.64-3.34)	0.62 ± 0.48^{b} (0.20-1.14)	**	0.005
Eosinophil (%)	2.81 ± 2.22^{a} (0.54-6.84)	1.12 ± 0.33^{ab} (0.74-1.54)	0.44 ± 0.31^{b} (0.00-0.94)	**	0.01
Basophils (%)	0.14± 0.00 (0.14-0.14)	0.15 ± 0.07 (0.04-0.24)	0.16 ± 0.11 (0.00-0.34)	NS	0.865

**= Significant at P<0.01; *= Significant at P<0.05; NS= Non significant

Table 4. Effects of K_3EDTA, heparin and sodium citrate on platelet count and its indices.

Parameters	Mean ± SD (Range)			Level of significance	P value
	K_3EDTA	Heparin	Sodium citrate		
Platelet (K/µL)	299.14 ± 364.76 (1.00-777)	319.86 ± 299.18 (30-715)	79.57 ± 177.92 (08-483)	NS	0.25
MPV (fL)	5.77± 3.95[a] (00-8.44)	6.44 ± 2.90[a] (00-8.24)	1.12± 2.96[b] (00-7.84)	*	0.02
PCT (%)	0.66 ± 0.74 (00-2.19)	0.24 ± 0.24 (00-0.58)	0.05 ± 0.13 (00-0.37)	NS	0.06

**= Significant at P<0.01; *= Significant at P<0.05; NS= Non significant

4. Conclusions

The three anticoagulants selected for this study have been successfully used in complete blood count and laboratory analysis. It was observed that Hct and MCV showed a marked decrease (P<0.01) in their value in citrated sample as compared to heparin and EDTA sample. The value of total leukocyte count (TLC) and absolute count of netrophil, monocyte and eosinophil showed a highly significant (P<0.01) decrease in citrated sample than that of EDTA and heparin sample. In differential leukocyte count the relative count of all parameters neutrophil, lymphocyte, monocyte, and eosinophil showed a significantly (P<0.01) lower level of their values in citrated sample. The difference anticoagulant had no significant effect on total platelet count and PCT (%) except the MPV (fL) value in citrated sample showed a significantly (P<0.05) lower value as compared to EDTA and heparinized samples. It can be concluded that samples treated with sodium citrate result in significant change in blood parameters, therefore it is better to use heparin and EDTA than sodium citrate.

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Conflict of interest

None to declare.

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