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Effect of anticoagulants and storage time on Red Blood Cell morphometry in domestic and pet animals

Tuli Dey[1], Sonnet Poddar[2]*, Jabin Sultana[3], Shubho Podder[4], Abdullah Al Faruq[2], A. S. M. Golam Kibria[2], A. S. M. Lutful Ahasan[2]

[1]Department of Medicine and Surgery, Chattogram Veterinary and Animal Sciences University, Khulshi – 4224, Chattogram, Bangladesh
[2]Department of Anatomy and Histology, Chattogram Veterinary and Animal Sciences University, Khulshi – 4224, Chattogram, Bangladesh
[3]Department of Physiology, Biochemistry and Pharmacology, Chattogram Veterinary and Animal Sciences University, Khulshi – 4224, Chattogram, Bangladesh
[4]Sheikh Sayera Khatun Medical College, Gopalgonj, Bangladesh

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***Corresponding Author**

Sonnet Poddar
sonnet@cvasu.ac.bd

ABSTRACT

The study was planned to determine the effect of anticoagulants and storage time on morphometry of RBCs in domestic and pet animal species. Blood from domestic animal species (cattle, sheep, goat, and dog) was collected in a vacutainer, blood bag treated with EDTA, and CPD anticoagulants stored at 4 ± 2° C for 4 weeks. The blood smears were done for different intervals up to 4 weeks of storage from EDTA and CPD treated blood. Staining of entire smears was carried out with wright stain. Blood figures were taken, and the diameter of RBCs was measured with the help of ImageJ software. The means diameter of RBCs of cattle, goat, sheep, and dog were 5.2±0.14, 3.34±0.13, 3.1±0.09, and 7.2±0.28 µm; respectively. Significant morphometric changes (decrease of the diameter of RBCs) were observed on the 14th day and 21st days with CPD and EDTA anticoagulants. The study showed less storage lesion of RBCs morphometry was found under using CPD anticoagulants in cattle, whereas EDTA showed less storage lesion of RBC morphometry in sheep and goats. It is recommended to use the specific anticoagulants for specific animal species, and the laboratory test should be performed within 7 days as the RBCs morphometry remain structurally good.

INTRODUCTION

Blood is the life-maintaining transport fluid that consists of numerous components such as Red Blood Cells, White Blood Cells, and Platelets. A variation on morphometry of Red Blood Cells (RBCs) in the animal is occurred and varies with age, breed, and sex (Aoki and Ishii, 2012; Zobra et al., 2011). The RBCs of cattle are biconcave-shaped without a nucleus (Wood et al., 2010). The RBCs of a horse are spherocytic shape and measured 5-6µm in diameter (Grondin and Dewitt, 2010). Canine has the largest RBCs (diameter 7-8 µm) with biconcave shaped among domestic animals (Rizzi et al., 2010). Ovine RBCs are the smallest (2-5µm diameter) mammalian RBCs (Simpraga et al., 2013). Caprian RBCs are fusiform-shaped with a diameter of 2.5-3.9 µm (Mbassa and Poulsen, 1992). The collected blood is treated with anticoagulants and stored at 4 ± 2° C for different laboratory tests (Baffour et al., 2013). Ethylenediamine tetraacetic acid (EDTA) salt is commonly used as an anticoagulant in blood samples (Kafka & Yermiahu, 1998). Longer storage of RBCs represents more storage lesions, and a smaller proportion of RBCs survived when they were transfused into animals. There also have an effect on the morphology of the blood cells, particularly in RBCs, due to longer storage of blood samples in EDTA tubes than normal. Several studies were conducted on morphometry of RBCs of common domestic animals species (Aoki and Ishii, 2012; Zobra et al., 2011; Rizzi et al., 2010), and fewer studies were conducted on

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the effects of anticoagulants and storage time on morphometry of RBCs (Walencik and Witeska, 2007; Berezina et al., 2002). Here the study is planned to determine the common domestic and pet animal species based on morphometry of RBCs and a determination of the effect of anticoagulants and storage time on morphometry of RBCs in common domestic and pet animal species.

MATERIALS AND METHODS

Study area and study population

The study was conducted at the Department of Anatomy and Histology, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. Common domestic and pet animal species – cattle, sheep, goat, and dog were considered for the study.

Sample collection and storage

Blood from common domestic animal species were collected in a vacutainer and also in a blood bag treated with EDTA and CPD anticoagulants, and these were stored at 4 ± 2° C for 4 weeks. Blood from different animals was collected from the Teaching Veterinary Hospital of CVASU and also from a different area of Chattogram metro.

Blood smear and staining

The blood smear with EDTA and CPD treated blood were performed for different intervals up to 4 weeks of storage. The staining of entire smears was carried out with classical wright stain.

Morphometric study of RBCs

The figures of RBCs were taken under an optical microscope at 100X magnification with immersion oil. The effect of anticoagulants and storage time on morphometry of RBCs in common domestic animal species were evaluated and recorded.

Analysis and interpretation of the results

Based on the RBCs morphometry of blood from different species at different storage time, the conclusion has been drawn out. Analysis of images (RBCs) has been analyzed with Image J software. Species were identified, and the changes in the morphometry of RBCs were evaluated. Statistical data were recorded and analyzed with STATA.

RESULTS

The means diameter of RBCs of cattle, goat, sheep, and dog were 5.2±0.14, 3.34±0.13, 3.1±0.09, and 7.2±0.28 µm; respectively (Table 1). All the images (RBCs) were analyzed with ImageJ software. The mean value (diameter of RBCs) are presented below (all value are expressed as mean±SD, p-value = <0.05).

Table 1: Diameter (µm) of RBCs of cattle, sheep, goat and dog

Anticoagulants	Species	1st Day	7th Day	14th Day	21th Day
EDTA	Cattle	5.2±0.14	4.8±0.46	4.2±0.15	3.8±0.21
	Goat	3.34±0.13	3.0±0.14	2.78±0.15	2.5±0.14
	Sheep	3.1±0.09	2.9±0.14	2.5±0.26	2.0±0.23
	Dog	7.2±0.28	6.5±0.23	6.0±0.23	5.0±0.24
CPD	Cattle	5.2±0.14	4.9±0.24	4.3±0.29	4.0±0.14
	Goat	3.34±0.13	3.1±0.09	2.7±0.32	2.3±0.05
	Sheep	3.0±0.14	2.7±0.32	2.4±0.28	2.0±0.23
	Dog	7.2±0.28	6.5±0.23	6.0±0.23	5.1±0.14

Table 1: Diameter (µm) of RBCs of male and female cattle, goat, sheep, dog

Animal	Sex	Anticoagulant	1st Day	7th Day	14th Day	21st Day
Cattle	Male	EDTA	5.1±0.14	4.7±0.15	4.1±0.29	3.7±0.21
	Female	EDTA	5.3±0.29	4.9±0.46	4.4±0.24	3.9±0.24
	Male	CPD	5.1±0.14	4.8±0.46	4.1±0.29	3.8±0.21
	Female	CPD	5.3±0.29	5±0.14	4.5±0.46	4.2±0.29
Goat	Male	EDTA	3.1±0.89	3.0±0.14	2.7±0.32	2.4±0.05
	Female	EDTA	3.5±0.13	3.2±0.23	2.9±0.14	2.6±0.32
	Male	CPD	3.1±0.09	3.0±0.14	2.5±0.14	2.2±0.23
	Female	CPD	3.5±0.13	3.3±0.14	2.9±0.14	2.4±0.05
Sheep	Male	EDTA	3.0±0.14	2.7±0.32	2.3±0.06	1.9±0.23
	Female	EDTA	3.3±0.13	3.1±0.89	2.7±0.32	2.1±0.23
	Male	CPD	2.9±0.14	2.6±0.32	2.3±0.06*	2.1±0.23
	Female	CPD	3.1±0.89	2.8±0.05	2.5±0.28	2.1±0.23
Dog	Male	EDTA	7.0±0.23	6.3±0.23	5.8±0.23	4.9±0.24
	Female	EDTA	7.4±0.28	6.7±0.23	6.2±0.14	4.9±0.24
	Male	CPD	7.0±0.28	6.2±0.14	5.9±0.28	5.1±0.14
	Female	CPD	7.4±0.28	6.8±0.23	6.2±0.14	5.2±0.14

Here, significant morphometric change (RBCs were shrinkage - a decrease of the diameter of RBCs) was observed at 14th and 21st days with EDTA anticoagulants compared to CPD anticoagulants in cattle. Significant morphometric changes (decrease of the diameter of RBCs) were observed at the 14th day and 21st days with CPD anticoagulant compared to EDTA anticoagulants in the goat. In sheep also, morphometric changes (decrease of the diameter of RBCs) were observed on the 14th day and 21st day with CPD anticoagulant compared to EDTA anticoagulants. In the case of a dog, there was a small morphometric change (decrease of the diameter of RBCs) was observed on 21st day with EDTA anticoagulant compared to CPD anticoagulants (Table 2).

The diameter of male RBCs of cattle was smaller than the female cattle (cow). Morphometric change (RBCs were shrinkage - a decrease of the diameter of RBCs) were observed at 14th and 21st days with EDTA anticoagulants compared to CPD anticoagulants in female cow (Table 2).

The diameter of female RBCs of a goat was larger than the male goat. Morphometric change (RBCs were shrinkage – a decrease of the diameter of RBCs) was observed at 14th and 21st days with CPD anticoagulants compared to EDTA anticoagulants in male goat (Table 3).

The diameter of female RBCs was larger than the male sheep. Morphometric changes (decrease of the diameter of RBCs) were observed on the 14th day and 21st day with CPD anticoagulant compared to EDTA anticoagulants in female sheep (Table 4).

The diameter of female RBCs was larger than male RBCs. A few morphometric changes (decrease of the diameter of RBCs) were observed at 21st days with EDTA anticoagulant compared to CPD anticoagulants (Table 5).

Abbrebriation: EDTA = Ethylenediamine Tetracetic Acid, CPD = Citrate Phosphate Dextrose, CVASU = Chattogram Veterinary and Animal Sciences University, RBCs = Red Blood Cells, SD = Standard Deviation.

DISCUSSION

The means diameter of RBCs of cattle, goat, sheep, and dog were 5.2±0.14, 3.34±0.13, 3.1±0.09, and 7.2±0.28µm; respectively. Those findings were similar to the previous result of Grondin and Dewitt (2010); Rizzi et al. (2010); Mbassa and Poulsen, (1992).

In the study of Rizzi et al. (2010), they found canine has the largest RBCs (diameter 7-8µm) with biconcave shaped among domestic animals. Simpraga et al. (2013), found - the RBCs of sheep are the smallest (2-5µm diameter) among mammalian RBCs. Caprian RBCs are fusiform-shaped with a diameter of 2.5-3.9µm, which was found by Mbassa and Poulsen (1992).

So, we may need to determine the common domestic and pet animal species based on morphometry of RBCs (diameter of RBCs).

The significant morphometric change (RBCs were shrinkage – a decrease of the diameter of RBCs) were observed at 14th and 21st days with EDTA (4.2±0.15µm, 3.8±0.21µm) anticoagulants compared to CPD (4.3±0.29µm, 4.0±0.14µm) anticoagulants in cattle. Those effects may occur in RBCs due to the longer storage of blood samples in EDTA and CPD tubes than normal. This study was supported by the previous study of Berezina et al. (2002) and Baffour et al. (2013).

Significant morphometric changes (decrease of the diameter of RBCs) were observed at 14th day and 21st days with CPD (2.7±0.32µm, 2.3±0.05µm) anticoagulant compared to EDTA (2.78±0.15µm, 2.5±0.14µm) anticoagulants in goat. This study was supported by the previous study of Berezina et al. (2002) and Baffour et al. (2013).

In sheep also, morphometric changes (decrease of the diameter of RBCs) were observed at 14th day and 21st days with CPD anticoagulant compared to EDTA anticoagulants. Here, we found, the diameter of the RBCs of sheep is smaller than other species. This finding was similar to the findings of Simpraga et al. (2013). They found the diameter of ovine RBCs is the smallest (2-5µm diameter) of mammalian RBCs. In the case of a dog, there were a few morphometric changes (decrease of the diameter of RBCs) were observed on 21st day with EDTA (5.0±0.24µm) anticoagulant compared to CPD (5.1±0.14µm) anticoagulants.

We noticed that longer storage of RBCs represents more storage lesion (shrinkage of RBCs), and a smaller proportion of RBCs survives when they go for delay laboratory tests or transfused into animals.

So, it is essential to determine the effects of anticoagulants based on storage time to decide on appropriate storage time for laboratory diagnosis of blood and transfusion in the animal and the common domestic and pet animal species identification.

For the study, we noticed that the RBCs morphometry might remain structurally suitable for 7 days. So, it is recommended to perform any laboratory diagnosis or transfusion of storage blood within 7 days. But it is better to use this blood immediately after drawn out from an animal.

CONCLUSION

Different anticoagulants may affect the shrinkage of RBCs morphometry for different storage times. The study showed less storage lesion on RBCs morphometry using CPD anticoagulants in cattle, whereas EDTA showed less storage lesion of RBC morphometry in sheep, goats. It is essential to know the effects of anticoagulants based on storage time to decide on performing laboratory diagnosis of blood and transfusion in an animal.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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