



Effect of storage on biochemical parameters of packed red blood cells of goats in citrate phosphate dextrose adenine/ saline adenine glucose mannitol*

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Citation: Anaz, S.R., Unny, M.N., Usha.N.P., George, A. and Venkatachalapathy, T.R. 2021. Effect of storage on biochemical parameters of packed red blood cells of goats in citrate phosphate dextrose adenine/ saline adenine glucose mannitol. *J. Vet. Anim. Sci.* 52(3): 257-261. DOI: <https://doi.org/10.51966/jvas.2021.52.3.257-261>

Received: 18.01.2021

Accepted: 24.02.2021

Published: 30.09.2021

Abstract

A study was conducted to assess the suitability of citrate phosphate dextrose adenine / saline adenine glucose mannitol as a storage media for packed RBCs of goats. Samples collected from ten apparently healthy goats were utilized for the study. Biochemical studies were carried out on day 0, 14, 28 and 42 days of storage using the parameters, viz. pH, glucose, potassium, malondialdehyde and reduced glutathione. The pH was stable throughout the study, whereas glucose showed significant reduction. Rest of the parameters increased significantly from 0th day to 42nd day. Based on the results, the storage media can be considered to be suitable for storing caprine packed RBCs.

Keywords: Biochemical parameters, blood transfusion, goat, packed RBCs, storage studies

With advances in veterinary transfusion medicine, blood component therapy is being increasingly practiced. Packed red blood cell (pRBC) transfusion is one of the most common component transfusion practiced in animal transfusions. As the need outweighs availability, storage of blood and its components is required for emergency transfusion procedures. Suitable storage media is a necessity with regard to storing of blood, as any storage lesion can adversely affect the viability of RBCs. Biochemical studies on caprine pRBCs stored in citrate phosphate dextrose adenine/ saline adenine glucose mannitol (CPDA/SAGM) are scarce. Hence, the present study was envisaged to assess the suitability of CPDA/SAGM as a storage media for caprine pRBCs.

*Part of M.V.Sc. thesis submitted by the first author to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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Materials and methods

Ten apparently healthy goats weighing 35 to 60 kg within an age range of 4 to 8 years were selected from University Goat and Sheep Farm, Mannuthy and other organized farms in Thrissur district. All the goats were dewormed and the health status of each animal was evaluated by physical examination and laboratory analysis including complete blood count, examination of blood smear and faecal sample. Whole blood units were collected from the selected animals using commercially available CPDA-SAGM blood bags under aseptic conditions. Each of these units was centrifuged at $5,000 \times g$ for 7 minutes, at 4°C using FTBC- 6100R blood bank refrigerated centrifuge. After centrifugation, plasma was extracted in empty bag using plasma extractor from primary blood bag and SAGM was added from the satellite bag using the valves. After separation, pRBCs with SAGM was stored at $4 \pm 2^\circ\text{C}$ in a dedicated refrigerator for 42 days and biochemical parameters analysed every two weeks from day 0 to day 42. Blood pH was estimated with portable Oakton waterproof pH meter. The level of GSH in erythrocyte suspension was determined as per Bain *et al.* (2016). This method is based on the development of a yellow colour when 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) is added to sulphhydryl compounds. Level of lipid peroxides in erythrocyte suspension was determined by estimating MDA using the method of Okhawa *et al.* (1979) as modified by Al-Azzawie and Alhamdani (2006). The supernatant glucose

estimation of packed RBC stored in SAGM was carried out using glucose estimation kit- GenX GLUCOSE-ML (GOD-PAP Trinders method). Potassium was estimated from the supernatant of packed red blood cells stored in CPDA/SAGM blood bag using ion selective electrode technology.

Results and discussion

The biochemical parameters assessed in this study were pH, glucose, potassium, MDA and GSH. Statistical analysis using repeated measures ANOVA was carried out to find out the changes in the variables at different period of time. In the cases where F-value was found significant, pair wise comparison was done by using least significant difference test. The mean values of biochemical parameters are as in table 1.

In this study, mean pH value decreased from day 0 to 42 during storage, though it was not significant. The mean value was in the range 7.18 ± 0.03 to 7.13 ± 0.03 . Hess and Greenwalt (2002), opined that pH value above 7.2 will favor 2, 3-bisphosphoglycerate production and might affect the ATP synthesis. The permissible lower pH limit for the stored blood was 6.65. Reduction in pH values occur as RBCs remain metabolically active, with glucose breakdown and anaerobic metabolism leading to production of lactate and hydrogen ions (Mudge *et al.*, 2004; Hess *et al.*, 2009). However, as the decline of pH was not found to be significant and was within the limits, the stored blood could be used for transfusion.

Table 1. Variation of bio-chemical parameters during storage

Variables	Day 0	Day 14	Day 28	Day 42	F-value (P-value)
pH	7.18 ± 0.0389	7.18 ± 0.0327	7.14 ± 0.0371	7.13 ± 0.0367	1.317 ^{ns} (0.289)
Glucose (mg/dL)	650.4 ± 18.45^a	607.5 ± 18.92^b	573.2 ± 22.09^c	535.5 ± 16.92^d	20.707 ^{**} (<0.001)
Potassium (mmol/L)	4.02 ± 0.22^d	6.96 ± 0.27^c	9.61 ± 0.39^b	13.15 ± 0.57^a	160.61 ^{**} (<0.001)
MDA ($\mu\text{mol/L}$)	10.47 ± 1.13^d	15.15 ± 1.36^c	20.59 ± 1.60^b	28.16 ± 1.76^a	33.342 ^{**} (<0.001)
GSH ($\mu\text{mol/g}$ of Hb)	0.02 ± 0.01^d	0.05 ± 0.01^c	0.08 ± 0.02^b	0.10 ± 0.02^a	46.62 ^{**} (<0.001)

** Significant at 0.01 level ($P < 0.01$); ns - Non-significant ($P > 0.05$)

Means having different superscripts differ significantly within a row

The mean value of glucose in supernatant reduced as the storage period increased. This is in agreement with Tavares (2013), who observed decrease in glucose values during storage from day 0 to 42. This reduction might be due to the consumption of glucose by the metabolically active erythrocytes as an energy source. However, it has been recorded that glucose consumption of caprine erythrocytes is low in comparison to other species. Higher ATP reserves of caprine erythrocytes may also result in non-decline of glucose levels (Kaneko *et al.*, 2008). Even at the end of storage period, significant reduction in glucose was noticed in the mean value from 573.2 ± 22.09 mg/dL on 28th day to 535.5 ± 16.92 mg/dL on 42nd day. This suggests that RBCs remained metabolically active and fit for transfusion. The excess glucose in the nutrient media at the end of storage period was easily balanced post transfusion by the liver (Fonesca *et al.*, 2018).

A significant increase was recorded in the supernatant potassium level during the study. On the 42nd day of storage, the supernatant mean potassium value was 13.15 ± 0.57 mmol/L, *vis-à-vis* day 0 value of 4.02 ± 0.22 mmol/L. During blood storage, a slow and continuous leakage of intracellular potassium to the plasma has been recorded. This has been considered to be associated with failure of Na⁺- K⁺ ATPase pump (Opoku-Okrah *et al.*, 2015). Species wise difference in intracellular potassium level has been reported (Sousa *et al.*, 2013). Opoku-Okrah *et al.* (2015), stated that plasma potassium level of blood stored in CPD solution can increase by 0.5-1.0 mmol/L per day. Extracellular potassium value can be used as a marker for assessing the quality of stored erythrocytes as the ATP depletion and improper refrigeration may result in elevation of potassium in ECF as suggested by Fonesca *et al.* (2018). Perusal of literature did not reveal any similar study for caprine pRBC storage with regard to potassium levels. The increase in potassium in stored blood of goat was slow and constant when compared with the increased potassium levels of stored canine and human blood. Further, the values of the present study can be used as reference values for caprine pRBC storage studies.

The mean MDA values increased significantly throughout the storage from 10.47 ± 1.13 μ mol/L (day 0) to 28.16 ± 1.76 μ mol/L (day 42). As MDA is an end product of lipid peroxidation, quantifying the MDA in stored blood was a useful marker to determine the extent of lipid peroxidation (Simsek *et al.*, 2006; Nazifi *et al.*, 2009; Pandey and Rizvi, 2011). Malondialdehyde formation indicated the loss of phospholipid from RBC membrane. Proportionating the MDA values, oxidative damage and resultant haemolysis can be assessed and viability of the cells in the storage media can be predicted (Fonesca *et al.*, 2018). Increase in MDA values on storage of pRBCs in SAGM of human blood has been reported (Chaudhary and Katharia, 2012). However in a similar study of human pRBCs, increase was found to be insignificant (Mustafa *et al.*, 2016). Similar studies with regard to stored caprine pRBCs are lacking. It has been suggested by Antosik *et al.* (2018) that addition of membrane interacting antioxidants like vitamin E analogue has beneficial effects in reducing lipid peroxidation level of RBCs stored in SAGM.

A significant increase in GSH was recorded with storage. Huyut *et al.* (2016) reported that MDA value and GSH value were negatively correlated. Reduced glutathione studies with respect to caprine pRBC stored in SAGM are lacking. Increase in GSH during the study period suggests antioxidant activity to manage the oxidative stress. According to Roback *et al.* (2011), the oxidised form of GSH is GSSG, which is not an antioxidant but it can be converted back to GSH by using NADPH derived from pentose phosphate pathway. It was also reported that supplementation of amino acid precursors can also stimulate GSH synthesis. Two other antioxidants reported were α - tocopherol and ergothioneine, which are obtained from the diet or some unknown mechanisms and not synthesized by the erythrocytes. These were relatively stable during the storage. Additive used in the study, SAGM contains mannitol which acts as an antioxidant by scavenging hydroxyl radical in various systems (Antosik *et al.*, 2018). Therefore, several factors influence the GSH values and no specific conclusion could be drawn with regard to the increase GSH values recorded in the present study.

Conclusion

The suitability of CPDA/SAGM as a storage media for caprine pRBCs in the present study was assessed using biochemical parameters. Based on biochemical studies, the storage media could be considered suitable for storing caprine pRBCs for 42 days.

Acknowledgement

The authors are thankful to the Kerala Veterinary and Animal Sciences University for providing the facilities needed for carrying out the research.

Conflicts of interest

There were no conflicts of interest reported by the authors.

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