REVIEW OF LITERATURE: Whole Blood Storage

Rapoport and Wing, 1947 examined the morphological, osmotic and chemical changes of erythrocytes stored in citrate, citrate glucose and ACD solutions, up to 50 days. Osmotic hemolysis increased throughout storage at a rapid rate in citrate and slowly in ACD. Storage of erythrocytes in ACD improved the ATP levels and ability of the cells to glycolyse and maintained shape of the erythrocytes. ACD solution was recommended for use as an efficient blood storage solution.

Gabrio et al., 1954a, Gabrio et al., 1954b, Gabrio et al., 1954c, Gabrio et al., 1955a, Gabrio et al., 1955b, Donohue et al., 1956 and Gabrio et al., 1956 elucidated the changes that occur in erythrocytes of whole blood stored in ACD at 4°C. Biochemical parameters (phosphates and glucose utilization), osmotic and mechanical fragility and post transfusion related survival were analyzed to study the erythrocyte storage lesion. The studies showed that changes occur in fragility, glucose utilization and carbohydrate metabolism during in vitro storage of blood. Young RBCs were found to be more susceptible to damage during storage than older RBCs. The damage during storage was found to be similar and unrelated to the life span of the erythrocytes in both rabbits and humans. The reversibility of the biochemical aspects of the storage lesion was demonstrated by introducing the stored erythrocytes back into circulation. The addition of adenosine to erythrocytes during storage proved beneficial in both rabbits and human blood by showing increased post transfusion viability, improved osmotic fragility, increased intracellular potassium levels, repletion of organic phosphates and increased glucose utilization. These studies elucidated that the reduction of the energy potential of the erythrocyte was mainly associated with erythrocyte damage during storage. The incubation of three week old stored erythrocytes with adenosine sustained the changes of the storage lesion for a further three weeks at 4°C. The addition of adenosine doubled the effective storage period when compared to storage in ACD. The utilization of inosine during storage improved the post transfusion survival of the erythrocytes after 6 weeks of storage.

Ponder and Barreto, 1957 studied the volume and shape properties of erythrocyte ghosts obtained from fresh heparinized blood and during storage in heparinized blood, ACD and ACD-inosine up to 82 days. The study showed that similar structural breakdown occurred in heparinized blood within 26 days, in ACD between 55-60 days and in ACD-inosine between 75-80 days of storage. The study emphasized the
need for increased efficiency of storage in order to decrease the rate of structural breakdown of erythrocytes.

Bartlett and Barnet, 1960 examined the accumulation of inorganic phosphates in erythrocytes of blood stored in ACD for 62 days. The levels of ATP, 2,3-BPG and fructose diphosphate (organic phosphates) rapidly decreased with storage and these observations correlated with the increased levels of inorganic phosphates. The levels of 2,3-BPG diminished within two weeks of storage. ATP levels initially decreased slowly, followed by a rapid decline as storage progressed. This study showed that the deterioration of erythrocytes with storage was associated with decrements in organic phosphates.

Bunn et al., 1969 investigated the effects of inosine on the storage of whole blood for 7 weeks. Blood stored in ACD and ACD-adenine showed increased oxygen affinity of hemoglobin within the first week of storage, which was attributed to the depletion of 2,3-BPG. Glutathione and hemoglobin levels were insignificant during the first three weeks of storage. Blood incubated and stored with inosine showed unaltered oxygen affinity for two weeks of storage and normalized 2,3-BPG and ATP, which in turn decreased the oxygen affinity of hemoglobin.

Celle, 1969 examined the alteration in deformability of the erythrocyte membrane of blood stored in ACD for 3 weeks. Deformability of erythrocytes decreased as storage progressed. Blood incubated with adenosine was associated with regeneration of ATP levels, which in turn restored the mean deformability and shape of the cells.

McNamara et al., 1971 studied the changes in physical properties of erythrocytes stored in ACD for 20 days at 4°C. The changes in hematocrit and fibrinogen were insignificant with storage. The screen filtration pressure and blood viscosity increased with storage indicative of lower deformability and changes in shape of the erythrocytes.

Palek et al., 1972 studied the dependence of erythrocyte ghosts on calcium and ATP during storage of whole blood in ACD during 6 weeks of storage. The study showed that the increase in calcium and depletion of ATP caused membrane protein changes, leading to loss of elasticity and permeability of erythrocytes. These changes, in turn cause the formation of ghosts of smaller size.
Bailey and Bove, 1975 studied the effect of storage on the chemical and hematological properties of blood stored up to 28 days in CPD. The study showed that plasma levels of potassium, Hb, lactate, lactate dehydrogenase (LDH) and ammonia increased while bicarbonate and dextrose reduced as storage progressed. The study concluded that biochemical changes in blood occurred due to processing and handling.

Schrier et al., 1979 reported the presence of a functional erythrocyte membrane storage lesion by measuring the levels of endocytosis in cells and resealed ghosts. Whole blood was stored for 6 weeks in CPD solution. Endocytosis was studied in intact erythrocytes by the influence of drugs, while endocytosis was induced by resealing of calcium, magnesium and ATP in the ghosts. Endocytosis was impaired when ATP levels were depleted and the restoration of ATP could not reduce the endocytosis.

Hamasaki et al., 1981 studied the effects of phosphoenolpyruvate as an additive in erythrocytes of blood stored in ACD for 28 days. ATP and 2,3-BPG levels increased in the groups incubated with phosphoenolpyruvate. The levels of ATP and 2,3-BPG decreased in the stored blood in ACD and ACD with phosphoenolpyruvate. The addition of phosphoenolpyruvate maintained ATP levels of stored blood for 14 days. The study emphasized the possibility of employing phosphoenolpyruvate for rejuvenation and for extending the shelf life of stored blood.

Beutler et al., 1982 studied the osmotic fragility of erythrocytes stored in CPDA-2 for 42 days with and without reinfusion in an iso-osmotic buffer. The lactate levels and osmotic fragility increased while the mean corpuscular volume decreased in stored non-reinfused erythrocytes. The reinfusion of the stored erythrocytes in an iso-osmotic buffer not only reversed the osmotic fragility of the stored erythrocytes, but also normalized the levels of osmotic fragility of the transfused cells.

Jarolim and Mircevova, 1982 assessed the effects of storage of whole blood in ACD (3-4 weeks) on the membrane fluidity of erythrocytes. The membrane fluidity of stored cells was higher than that of fresh erythrocytes, which was attributed to loss of functional complexes in the membrane, as storage progressed. The rejuvenation of the erythrocytes with glucose and inosine decreased membrane fluidity. Membrane fluidity was found to be associated with ATP levels during storage.
Latham et al., 1982 analyzed biochemical parameters during storage of whole blood in CPDA-1 for 35 days. The study’s results showed similarities with the earlier study of Bailey and Bove, 1975 which showed variations in plasma characteristics as storage progressed. The study suggested that assessment of the storage lesion through biochemical characteristics of plasma showed cell damage throughout storage.

Wiley et al., 1982 studied the effects of cold storage of erythrocytes of whole blood on calcium permeability. Whole blood was stored in CPD, ACD, CPDA or heparin for 42 days. The ATP levels reduced in ACD and CPD but fell slowly in CPDA. The calcium permeability increased irrespective of the storage solution used and did not affect sodium or magnesium permeability, indicating an ATP independent calcium leak develops during cold storage.

Estep et al., 1984 demonstrated that the addition of the plasticizer DEHP (di-(2- ethylexyl) phthalate) assisted in reverting the normal discoid morphology of stored erythrocytes, but did not have significant effects on the levels of ATP, 2,3-BPG, sodium or potassium concentrations. DEHP prevented the degradation of the RBC membrane which occurs during whole blood storage in CPD for 35 days.

Lorand and Michalska, 1985 demonstrated the response of erythrocytes of stored blood to calcium stress. Calcium stress induces proteolytic responses and cross-linking reactions. Whole blood stored in CPDA-1 for 14 days was subjected to calcium stress. The proteolytic response of the erythrocytes to calcium stress was elicited in only the fresh cells in Band 3 and glycoporphin, while the protein cross linking was observed in both fresh and stored erythrocytes. The study elucidated the possibility of utilizing loss of proteolytic response to study erythrocyte damage during storage.

Wolfe et al., 1986 studied the defects that occur in the membrane skeleton of stored blood. Blood stored in CPD for 6 weeks was used to analyze the membrane composition and protein interactions in the erythrocytes. Storage related injury to membrane was associated with decline in the spectrin-actin complexes leading to weakening of the membrane skeleton and decreased survival of erythrocytes.

Vora, 1988 explored the use of various additives on the levels of ATP and 2,3-BPG in erythrocytes of blood stored in CPD or CPDA-1 for 6 weeks. Oxalate, glyoxalate,
ethyl oxaloacetate and L-phenylalanyl-L-alanine maintained the 2,3-BPG levels during storage.

Rocchigiani et al., 1989 investigated the changes in erythrocyte density during storage of whole blood in ACD or CPD-adrenaline for 30 days. The erythrocytes were separated by density gradient centrifugation and analyzed for enzyme activity. The density of the erythrocytes increased after storage in both additive solutions. Glucose-6-phosphate dehydrogenase was used as a marker of erythrocyte age and the decline of enzyme activity with density was less marked after storage. The study states that aging of erythrocytes and density modifications are not related.

Seidl et al., 1991 developed a new blood storage container (PL 2209, plasticized with butyryl-n-trihexyl-citrate BTHC) and evaluated its efficacy in vitro and in vivo. Whole blood and red cell concentrates in CPDA-1 were stored for 42 days at 4°C. The measurements of lactate production, glucose consumption, sodium, potassium, ATP, hemolysis and in vivo recovery showed that the BTHC plasticized bags were effective in allowing 35 days of storage and could be employed instead of the DEHP bags.

Schleuning et al., 1992 studied the activation of the complement cascade during storage of blood under normal blood bank conditions. Whole blood collected in CPDA-1 and leucoreduced blood in CPDA-1 were stored up to 21 days. The activation of the complement system was carried out by radioimmunoassay quantifications of complement derived antigens. The study showed that during storage, the presence of leucocytes and storage in plastic bags caused increased levels of proteinases, which in turn activated the complement cascade, as seen in high levels of the complement-derived antigens.

Oyewale, 1993 and Oyewale et al., 2011 explored the effects of storage on the osmotic fragility of mammalian erythrocytes (human, rat, rabbit, cattle, sheep, pig, camel, donkey and goat). Blood was collected in EDTA and stored at 10°C for 24 hours. Significant changes in osmotic fragility were observed in all mammalian erythrocytes, except sheep. The osmotic fragility is affected by pH and temperature due to changes in erythrocyte proteins and lipids. The study stresses the determination of osmotic fragility within 24 hours of blood collection.
Aslan et al., 1997 studied the effects of storage on erythrocyte antioxidant enzymes and lipid peroxidation, during whole blood storage in CPDA-1 for 31 days at 4°C. An increase in the levels of malondialdehyde were observed between days 3 to 19, while superoxide dismutase and glutathione peroxidase reduced from day 9 and 13 respectively, up to day 27 of storage. This study also studied the changes in malondialdehyde in the plasma of stored blood.

Jozwik et al., 1997 investigated the levels of antioxidant defense in erythrocytes and plasma during whole blood storage in CPDA-1 for 25 days. The study showed a depletion in glutathione-dependent antioxidant systems (glutathione reductase, glutathione and glutathione-S-transferase) and superoxide dismutase in erythrocytes and total antioxidant capacity in plasma. The authors suggested twelve day storage period as a safe storage limit.

Hovav et al., 1999 elucidated the changes that occur in red cell shape and aggregability during storage, when whole blood was stored in CPDA-1 for 42 days. Erythrocyte shape changed from normal discocytes to echinocytes. The aggregability of erythrocytes increased but levels of plasma fibrinogen decreased with storage. The erythrocytes did not show increased aggregability when stored in autologous plasma. Changes in shape and aggregability in the erythrocytes during storage was responsible for variations in flow properties and functioning of erythrocytes after transfusion.

Turkes et al., 2003 studied the alterations in membrane proteins and phospholipids by spin label methods using whole blood, aged erythrocytes and artificially aged erythrocytes stored under blood bank conditions for 60 days. The study showed that erythrocytes are impaired at 37°C at faster rates than those stored at 4°C and that the oxidative damage of the membrane is responsible for structural and functional changes in stored erythrocytes.

Agarwal et al., 2005 studied the prospects of employing gamma irradiation before transfusion of blood and its components. Whole blood stored under blood bank conditions was irradiated and stored up to 21 days. An increment in hemoglobin, potassium and lactate dehydrogenase was observed in the irradiated samples, illustrating the requirement of studying the post transfusion effects of transfused, irradiated blood.
Marjani et al., 2007 investigated the effect of storage on lipid peroxidation and antioxidant enzymes in erythrocytes of blood stored in CPDA-1 for 35 days. Levels of potassium, malondialdehyde and lactate dehydrogenase activity increased in the plasma while erythrocyte enzyme activities (superoxide dismutase and glutathione peroxidase) and count decreased as storage progressed. The free radical damage occurred during days 7-11 and the initial hemolysis was due to increased lipid peroxidation and decline in antioxidant enzymes. The authors suggest the supplementation of donors at least a week before donation to ensure improved viability and longevity of the erythrocytes.

Hampson, 2008 studied the stability of carboxyhemoglobin in blood stored for 28 days with sodium heparin at room temperature and under refrigeration. There was no effect of temperature on carboxyhemoglobin levels throughout storage, ensuring stability of carboxyhemoglobin for analyses up to 28 days of storage.

Rigamonti et al., 2008 showed that erythrocytes of stored rat blood restored the cerebral oxygen delivery to the baseline levels, but these levels were lower than the levels achieved by transfusion of fresh blood. Rat blood was stored in CPDA-1 and transfused within 1 hour or after 7 days. The storage of blood limited the ability of the erythrocytes to deliver oxygen to the brain tissue.

Gulliksson and van der Meer, 2009 studied the effects of temperature and time of overnight storage of whole blood in CPD before preparation of components. The study compared the differences in storage containers along with overnight storage at room temperature and refrigerated conditions. Lower levels of potassium, 2,3-BPG and pH were observed in erythrocytes prepared from blood stored overnight at room temperature, while storage at 2-6°C showed increased ATP and hemolysis. Erythrocytes processed after overnight storage of 18-24 hours showed significant differences in in vitro parameters when compared to blood processed within 8 hours, with no differences between the storage containers used.

Li et al., 2010 examined the role of reactive carbonyl species in blood viscosity and storage lesion. Whole blood stored in CPD for 20 days showed increases in blood viscosity, plasma carbonyls and membrane protein carbonyls and decrements in thiol concentration. The carbonyl species induce structural alterations in erythrocytes leading to reduction in the efficacy and safety of blood transfusion.
Karger et al., 2012 studied the deformability of erythrocytes in leucocyte depleted whole blood stored in CPDA-1 for 49 days. ATP and erythrocyte deformability were measured and showed that ATP levels decreased and deformability at lower shear stress increased with storage. However, at higher shear stress, deformability decreased, proving that ATP was not a valid marker for RBC deformability or in vivo survival of stored erythrocytes.

Qazi et al., 2012 demonstrated the changes in levels of nitric oxide derivatives in erythrocytes under different storage conditions. Whole blood was collected and stored in CP2D and leucoreduced and non-leucoreduced erythrocytes were stored in AS3 for 42 days either in room air or in an argon chamber. Nitrite concentrations increased while nitrate concentrations remained stable throughout storage. The nitric oxide bioactivity was found to occur through nitrate nitrite pathway.

Rajashekharaiah et al., 2012 stored rat blood in CPD for a period of 14 days and studied the oxidative stress markers during storage. Hemolysis, malondialdehyde and advanced oxidation protein products showed changes during storage. The authors concluded that the erythrocytes were capable of protecting themselves against the oxidative stress induced through storage for up to two weeks.

Deyhim et al., 2014 showed that antioxidant enzymes (superoxide dismutase and glutathione peroxidase) decreased significantly on the 14th day of storage when whole blood was stored for a period of 35 days in CPDA-1.

Owusu-Ofori et al., 2015 studied the effects of pathogen reduction technology (using riboflavin and UV light) on the safety of stored blood. Whole blood stored in CPD for 21 days at 4°C was monitored for cell quality with and without pathogen reduction treatment. The pathogen reduction treatment reduced the levels of malarial parasites and maintained blood quality.

Verma et al., 2015 investigated the changes in biochemistry during storage of blood in CPDA-1 for a period of 21 days. Variations in the biochemical parameters were observed in ions, pH and enzymes, as a result of storage. The study emphasized the need to transfuse fresh or blood stored for less than 7 days for safe transfusion.

Dahiya et al., 2016 assessed the effects of pre-storage gamma radiation of the lipid profile of human blood stored in CPDA-1 for 21 days. The study showed that low density cholesterol increased in pre-storage irradiated samples on days 14 and 21. The
study elucidated the need to transfuse non-irradiated fresh or stored blood (up to 14 days) to patients with cardiac risk.

de Wolski et al., 2016 developed a murine model to identify the metabolic changes that occur in erythrocytes during storage of whole blood in CPDA-1. The study showed that oxidative stress and peroxidation of lipids mainly affected the erythrocytes during storage and hence, had an effect on circulation post storage.

Obisike, 2016 analyzed the variations in serum electrolytes and pH when human blood was stored in CPDA-1 for a period of 35 days under standard blood bank conditions and in a traditional refrigerator. Sodium, pH, chloride and bicarbonate decreased while potassium increased as storage progressed. The variations during storage in a traditional refrigerator were greater than the standard blood bank conditions. Hence, this study emphasized the need to follow the standard techniques of blood storage prior to transfusion.

Ugurel et al., 2017 studied the erythrocyte deformability during storage by applying mechanical stress. Whole blood stored in CPDA-1 for 35 days was metabolically depleted and analyzed for their response to mechanical stress. The stored erythrocytes showed greater impairment in deformability when compared to the metabolically depleted blood. The impairment in deformability was in relation to the storage time and the deformability varied inversely with levels of shear stress. The study elucidated the need to assess the storage time of blood before transfusion.

Erythrocyte Storage

Studies on the storage of erythrocytes in various additive solutions have also been carried out.

d’Almeida et al., 2000 explored the similarities in rat and human storage lesion through biochemical and functional parameters. Rat and human whole blood were stored in CPDA-1 at 4°C for a period of 29 days. Biochemical parameters such as ATP, 2,3-BPG, pH, LDH, lactate, Hb, hematocrit and K⁺; membrane deformability and 24-hr post transfusion viability were determined. The stored RBCs were also treated with a rejuvenating solution to assess the regeneration of ATP and 2,3-BPG. The study showed similarities in depletion of 2,3-BPG levels of rat and human erythrocytes. ATP levels were found to drop rapidly, while the rejuvenation solution regenerated only ATP in the rat samples. The study emphasized that the storage lesion
formed in rat over 1 week was correlated to the storage lesion formed in humans over 4 weeks.

Various aspects of the erythrocyte storage lesion have been studied by the group of Hess J.R. Hess et al., 2000 demonstrated that the addition of phosphate elevated ATP in accordance with increased volumes of additive solutions. Hess et al., 2001 studied the effects of an experimental additive solution (EAS -67) on erythrocytes and showed that storage for 11 weeks with mannitol maintained hemolysis levels to less than 1% and showed greater than 75% post transfusion recovery. Hess et al., 2002 explored the effects of utilizing alkaline storage solutions on preserving 2,3-DPG levels. The study showed that at high pH, 2,3-BPG was produced at the expense of ATP. This study concluded that the addition of surplus phosphate and alkaline pH, together could maintain the levels of ATP and 2,3-BPG in erythrocytes during storage. Hess et al., 2003 elucidated the positive effects of EAS-76 in prolonging erythrocyte storage up to 12 weeks as observed in lower hemolysis and microvesiculation and higher ATP levels. Hess et al., 2005 assessed the difference between laboratories in determination of ATP, 2,3-BPG and hemolysis of AS-1 stored erythrocytes and found that errors in measurements occurred between laboratories. Hess et al., 2006 studied the effects of employing buffering and dilution of the additive solutions in improving characteristics of stored erythrocytes. The study revealed that a combination of buffering and dilution reduced hemolysis and enhanced post transfusion survival of erythrocytes.

Cancelas et al., 2015 demonstrated that erythrocytes stored in AS-7 up to 56 days showed increased post transfusion recovery and lower hemolysis and microvesiculation in comparison to erythrocytes stored in AS-1.

Paglia et al., 2016 studied the effects of storage on SAGM stored erythrocytes in terms of metabolic markers. The study showed three distinct metabolic phases (i) days 0-10 (ii) days 10-18 and (iii) after day 18. The study also identified 8 biomarkers (lactic acid, nicotinamide, 5-oxoproline, xanthine, hypoxanthine, glucose, malic acid and adenine) which were highly correlated with the metabolic age of erythrocytes.

Jani et al., 2017 studied the biochemical and mechanical characteristics of rat erythrocytes stored in CPDA-1, AS-1 and AS-7 for 42 days. Similar variations were observed in all three additive solutions up to 2 weeks of storage. AS-7 showed
beneficial effects on the erythrocytes in comparison to CPDA-1 and AS-1 by reducing acidosis, hemolysis and lactate; preventing loss of potassium, reduction in deformability, ATP and 2,3-BPG and maintaining aggregability and viscosity of the erythrocytes.

Larson et al., 2017 showed that phosphatidylethanolamine (PE), a phospholipid found primarily on the inner leaflet of the erythrocyte membrane, is increasingly exposed in the outer leaflet during AS-1 storage of erythrocytes. This exposure was correlated with hemoglobin loss and microvesiculation.

**Antioxidants and Erythrocytes**

The effect of various antioxidants on erythrocytes have been studied through supplementation and during storage.

**Vitamin C**

Wood and Beutler, 1973, studied the effects of periodic mixing on maintenance of 2,3-BPG levels during storage. Whole blood was stored in CPDA and ACDA as controls and CPDA-sodium ascorbate (5 mM) and BAGPM (bicarbonate adenine glucose phosphate mannitol) as experimentals for 42 days. Half of the bags were mixed rigorously for five days every week. The frequent mixing of the bags yielded higher glucose, ATP and 2,3-BPG. CPDA-sodium ascorbate erythrocytes showed greater viability after 28 days while BAGPM erythrocytes showed increased viability after 42 days.

Wood and Beutler, 1974 studied the effects of ascorbate and dihydroxyacetone on ATP and 2,3-BPG levels of stored blood. Whole blood was stored in CPD with ascorbate, dihydroxyacetone or a combination of both. The levels of 2,3-BPG were maintained in the experimental groups at different levels of pH. The study showed the successful maintenance of the 2,3-BPG levels during 28 days of storage.

Moore et al., 1981 developed additive solutions for improvement of the erythrocyte storage. Erythrocytes were stored in CPD, CPDA-1 and CPDA-2 for 42 days with ascorbate-2-phosphate (1.4 -4.2mM). ATP levels were maintained by utilizing glucose and adenine in the storage solutions. The addition of ascorbate-2-phosphate additionally helped in the maintenance of 2,3-BPG levels, hence demonstrating the potential of improved erythrocyte storage.
Moore et al., 1988 studied the effects of an experimental additive solution containing ascorbate-2-phosphate as an additive during 42 days of erythrocyte storage. The levels of 2,3-BPG, ATP, glucose and pH varied as the storage temperatures differed (2.5°C and 5.5°C). Ascorbate helped in preserving the levels of 2,3-BPG through oxalate.

Knight et al., 1993 elucidated the effects of antioxidants (ascorbic acid and dimercaptosuccinic acid- 10mM) and metal chelators on lipid peroxidation of erythrocytes during storage. Whole blood was stored in CPDA for 19 days and malondialdehyde was assessed. The antioxidants increased the lipid peroxidation over controls, which could be attributed to the prooxidant activity of ascorbic acid.

Kurup et al., 2003 investigated the effects of ascorbic acid (0.077mM) as a component of CPDA during whole blood storage and SAGM during erythrocyte storage. The additive solution could effectively maintain the levels of 2,3-BPG by modulating pH during storage.

Zan et al., 2005 studied the effects of vitamin C on whole blood stored in CPD at 25°C for 6 days. The study showed that superoxide dismutase and ATP increased, while malondialdehyde, plasma potassium and superoxide radical levels decreased with the addition of vitamin C.

Raval et al., 2013 studied the effects of ascorbic acid on whole blood derived, pre-storage leucoreduced erythrocytes stored in AS-5 for 56 days. Ascorbic acid from 2.93-8.78mM was employed as additive in this study. Mechanical fragility, hemolysis, pH, methemoglobin and erythrocyte gases were estimated in the stored erythrocytes. Hemolysis and mechanical fragility decreased as the concentrations of ascorbic acid increased. The biochemical parameters remained similar in the controls and ascorbic acid samples. The study concluded that ascorbic acid could protect the erythrocytes from oxidative stress during storage and thus, can be explored as a component of additive solutions.

Stowell et al., 2013 studied the effects of ascorbic acid (3.6-72.1mM) on murine erythrocytes stored in CPDA-1 for 14 days. The stored erythrocytes showed decreased post transfusion viability and increased microparticle formation. The addition of ascorbic acid up to 10.8mM reduced microparticles and alloimmunisation and increased post transfusion viability. The study elaborated on the need for further evaluation of the biochemical changes of erythrocytes when stored with ascorbic acid.
Pallotta et al., 2014 studied the metabolomics of erythrocyte units with vitamin C and N-acetylcysteine during storage. Whole blood was collected in CPD, following which erythrocyte units were prepared in SAGM and stored for 42 days. Ascorbic acid (0.23 mM), N-acetylcysteine (0.5 mM) or a combination of both were used with SAGM as storage solutions for the red cell units. The antioxidants were able to promote glutathione homeostasis, which in turn decreased hemolysis, malondialdehyde and oxidation of glutathione and prostaglandins. The antioxidants combated oxidative stress but at the expense of glycolytic metabolism.

Czubak et al., 2017 explored the effects of sodium ascorbate (5 µM to 3 mM) and a mixture of sodium ascorbate and trolox (25 µM and 125 µM) on RBCs stored for 20 days. Sodium ascorbate elevated the total antioxidant capacity and inhibited lipid peroxidation, but did not protect against hemolysis. The addition of trolox and sodium ascorbate inhibited hemolysis, glutathione depletion, lipid peroxidation and increased the total antioxidant capacity. The prospects of utilizing vitamin C during storage, in combination with trolox has been elucidated in this study.

Sanford et al., 2017 investigated the effects of vitamin C on erythrocytes stored in SAGM for 56 days. The addition of vitamin C, both reduced and oxidized (up to 3 mM), protected the erythrocytes from oxidative damage by reducing osmotic fragility and increasing the antioxidant capacity and membrane integrity during storage. The study showed that oxidative stress played a major role in the formation of the storage lesion and vitamin C in combination with other antioxidants could improve storage of erythrocytes.

**Curcumin**

Curcumin increases intracellular GSH and regulates antioxidant enzymes (Sreejayan and Rao, 1993; Shishodia et al., 2005). Curcumin inhibited reactive oxygen species (ROS) generation (Kunchandy and Rao, 1990) and decreased osmotic fragility (Kempaiah and Srinivasan, 2002) and hemolysis (Somparn et al., 2007) in rat erythrocytes. Banerjee et al., 2008 studied the effects of curcumin on AAPH induced hemolysis of RBCs and showed a concentration dependent decrease in TBARS and hemolysis on treatment with curcumin.

Storka et al., 2013 investigated the effects of liposomal curcumin on morphology of erythrocytes of blood stored in EDTA for 4 hours. Increases in mean cellular volume
and echinocyte formation were observed after 30 minutes of incubation with 1, 10 and 100µg/mL of liposomal curcumin. This study showed the dose-limiting toxicity of curcumin on erythrocytes.

**Caffeic acid**

Caffeic acid inhibited hemolysis (Ganjii *et al.*, 2012), increased the antioxidant potential of plasma and erythrocytes (Lekse *et al.*, 2001), and protected erythrocytes from UVB radiation (Hsieh *et al.*, 2005) and toxicity (Abdallah *et al.*, 2012).

Huyut *et al.*, 2016a and Huyut *et al.*, 2016b studied the effects of caffeic acid (30µg/mL), tannic acid (15µg/mL) and resveratrol (30µg/mL) on erythrocytes of blood stored in CPD for 28 days. Erythrocyte lipid peroxidation and sensitivity to oxidation increased, while glutathione, glutathione peroxidase and catalase decreased in controls. The addition of tannic acid addition showed increased lipid peroxidation on days 21 and 28 of storage. Resveratrol and caffeic acid successfully preserved the antioxidant defenses and reduced lipid peroxidation throughout storage.

**Vitamin E**

Devi *et al.*, 1998 studied the effect of vitamin E (200µg/mL) on storage of blood in bags with DEHP, by analyzing antioxidant enzymes and lipid peroxidation levels. The effect of DEHP on erythrocytes during whole blood storage in CPDA was compared to storage in glass bottles. DEHP increased the lipid peroxidation and decreased potassium and hemoglobin during storage. Changes in lipid peroxidation were reversed by the addition of vitamin E. This study showed that the levels of DEHP and vitamin E were inversely related. The DEHP in bags was taken up by the erythrocytes and was localized in the erythrocyte membrane, hence accounting for membrane stabilization of the erythrocytes.

Begum and Terao, 2002 showed the protective effects of tocotrienol (50µM) against free radical-induced inhibition of deformability, while Claro *et al.*, 2006 showed that vitamin C (0.1-90mM) and E (0.1-80mM) contributed to a decrease in oxidative stress in erythrocytes after the action of phenylhydrazine hydrochloride.

Silva *et al.*, 2017 explored the effects of vitamin E as nanoemulsions on stored red blood cells. RBC units collected in CPD-SAGM were stored up to 35 days at 4°C with 0.2mg/mL vitamin E. The erythrocyte elasticity and reactive oxygen species levels were monitored throughout storage. Vitamin E could reduce ROS but did not
vary the elasticity of erythrocytes during storage. This could be attributed to the property of vitamin E to act on lipids, but not on protein oxidation. Hence, this study suggested the combination of vitamin E with protein protecting antioxidants to ensure better storage of erythrocytes.

**L-carnitine**

L-carnitine and its esters are found in large amounts in erythrocytes (Cooper et al., 1988). L-carnitine and its short-chain esters have demonstrated a positive effect on the stability of erythrocyte membranes under various adverse conditions and they also affect the molecular dynamics of erythrocyte membrane components (Kobayashi et al., 1989; Watanabe et al., 1989; Arduini et al., 1990; Arduini et al., 1993; Arduini et al., 2007; Wanic-Kossowska et al., 2007).

Arduini et al., 1997 studied the effects of L-carnitine (5mM) on erythrocyte integrity during storage of erythrocytes in AS-3 solution for 42 days. The addition of L-carnitine during erythrocyte storage caused an increase in the uptake of L-carnitine and generation of long chain acylcarnitine. These processes were related to low levels of hemolysis and high levels of ATP. The transfusion of these erythrocytes also showed greater *in vivo* viability when compared to controls, hence illustrating the positive effects of L-carnitine on blood storage.

Sweeney and Arduini, 2004 studied the effects of L-carnitine (5-15mM) on erythrocytes stored for 42 days in AS-3, with and without gamma irradiation. The study showed that *in vivo* survival and ATP levels improved, while hemolysis and supernatant hemoglobin reduced in the L-carnitine-treated RBC. A concentration of 15mM was found to be beneficial to erythrocytes during storage.

Arduini et al., 2007 explored the effects of L-carnitine (5mM) on the age related properties of erythrocytes stored in SAGM for 8 weeks. The study described the protective effect of L-carnitine on older erythrocytes as it prevented loss of potassium ions, thereby reducing osmotic swelling of the erythrocytes.

**Other antioxidants**

Sekeroglu et al., 2012 studied the effects of melatonin and propofol in erythrocytes during blood stored in CPDA-1 for a period of 28 days. The study showed increases in lipid peroxidation and decreases in antioxidant enzymes and glutathione during storage, which were reversed by the addition of melatonin. The effects of propofol
were observed only on glutathione and glutathione peroxidase, but not on the other enzymes and lipid peroxidation.

Sirdah et al., 2013 studied the effects of taurine on complete blood count during blood storage in EDTA for seven days. The study showed that complete blood count varied based on temperature, time and taurine concentration.

Ozcelik, 2014 evaluated the effects of melatonin on human erythrocytes during whole blood storage in CPD for three weeks. Mean corpuscular volume, platelet volume, acidity, oxidative damage, malondialdehyde and osmotic fragility increased and superoxide dismutase decreased with storage. The addition of melatonin reduced malondialdehyde and increased pH and superoxide dismutase. The study confirmed the primary role of oxidative stress in formation of the storage lesion and supported the storage of whole blood with melatonin for up to 21 days.

Zbikowska et al., 2014 evaluated the effects of quercetin on irradiation and storage induced oxidative damage in erythrocytes. Quercetin (2-50µM) was added to erythrocytes prepared from whole blood stored in ACD and lactate dehydrogenase, lipid peroxidation and glutathione levels were estimated. The addition of quercetin on irradiation and storage induced oxidative stress did not prove beneficial to erythrocytes.

Studies on blood storage and different additive solutions have improved blood storage conditions. The literature review shows that oxidative stress contributes significantly to the formation of the storage lesion. Studies have focused on whole blood as well as erythrocyte storage, with emphasis on the effect of storage on hemoglobin, biochemical parameters (ATP, 2,3-BPG, potassium, LDH), hemolysis and osmotic fragility. There are very few studies on the effects of oxidative stress during blood storage and these studies have focused on antioxidant enzymes, lipid peroxidation and protein oxidation. A comparative study between rat and human erythrocyte storage lesion (d’Almeida, 2000) also focused on the biochemical modifications during storage. Hence, the continuous assessment of the effects of oxidative stress on erythrocytes of stored blood needs to be explored.

Studies on antioxidants as additives of blood storage solutions are few and have focused on biochemical markers of the storage lesion. The effects of vitamin C on erythrocytes during storage have been elucidated with importance on ATP, 2,3-BPG,
lipid peroxidation, hemolysis and fragility in erythrocyte and blood storage. There exists limited literature on the effects of curcumin, caffeic acid, vitamin E and L-carnitine on erythrocytes during blood storage. However, a comprehensive understanding of the effects of these antioxidants as additives in blood storage solutions has not been explored. Further, there are few studies on the effects of antioxidants on erythrocytes of stored blood. This opens up avenues for exploration of these antioxidants as additives in blood storage solutions.

Hence, our study is aimed towards (i) understanding the influence of oxidative stress during storage and (ii) exploring the utilization of antioxidants as additives on rat and human erythrocytes of stored blood.
OBJECTIVES

- To assess the oxidant levels of erythrocytes during storage in terms of hemolysis, lipid peroxidation and protein oxidation products.
- To determine the antioxidant status during storage.
- To evaluate the role of antioxidants as components of additive solutions during storage.
- To determine the optimum concentrations of the antioxidants (Vitamin C, Curcumin, Caffeic acid, Vitamin E, L-Carnitine) which may prolong the shelf life of stored blood.

The above objectives were achieved by assessing the following:

1. Morphology and Erythrocyte count
2. Hemoglobin
3. Reactive oxygen species and Superoxides
4. Antioxidant enzymes (Superoxide dismutase, Catalase and Glutathione peroxidase)
5. Total antioxidant capacity (CUPRAC-BCS and Antioxidant activity) and Ascorbic acid
6. Lipid peroxidation products (Conjugate dienes and Malondialdehyde)
7. Protein oxidation products (Advanced oxidation protein products, Sulphhydryls and Band 3)
8. Hemolysis and Osmotic fragility
9. Glucose