ABSTRACT

Though refrigeration of whole blood and packed red blood cell (PRBC) in 2°-6°C provide longer shelf life up to 35-42 days (depending on anticoagulants), question remain about their efficacy at the end of storage time. Moreover, storing whole blood and PRBC for such a long time is reportedly associated with various changes in the red cell and plasma which are collectively known as 'storage lesion'. It was a matter of debate in the scientific community whether transfusion of older blood unit carries any risk to the recipient. This review article tries to focus on the various effect of storage on red cell and their potential involvement in the clinical outcome of recipient in the view of recent exploration in this field.

Keywords: Fresher Blood, Transfusion.

Introduction

Blood transfusion plays a pivotal role in surgery, chemotherapy, intensive care, and in treating thalassaemias, aplastic anaemia and various types of refractory anaemias. A safe and adequate blood supply need proper storage of blood and blood components so that it can be available at the time of need without compromising blood quality. The technique of blood preservation that is applied today is the result of cumulative effort of scientists of several nations worked over a span of 100 years. Their work mainly targeted to increase the life span of RBC outside the human body resulting in the storage duration of maximum 42 days for RCC with modified storage solution, improved blood bag and processing procedures. Though this storage duration fulfils the criteria of survival of 75% of transfused red cell beyond 24 hours and the expected haemolysis of the unit less than 1% at the end of storage time, question remains about their functional capability and effect exert on recipient.1

It is an established fact that RBCs undergo structural, morphological, biochemical and functional deterioration of RBC outside the human body resulting in the storage duration of maximum 42 days for RCC with modified storage solution, improved blood bag and processing procedures. Though this storage duration fulfils the criteria of survival of 75% of transfused red cell beyond 24 hours and the expected haemolysis of the unit less than 1% at the end of storage time, question remains about their functional capability and effect exert on recipient.1

It is an established fact that RBCs undergo structural, morphological, biochemical and functional deterioration
during storage. Reduced post transfusion survival, decreased ATP, 2,3 DPG, Na+–K+ gradient, and membrane changes are the major of these changes which may affect the post transfusion mortality and morbidity of recipients despite the reversibility of many of these storage lesions. Now concerns have been raised in the last two decades by several authors regarding safety of transfusion of blood unit at their end of storage time.

In Bangladesh, blood transfusion is carried out according to ‘Safe blood transfusion act 2008’ which clearly states that blood collected in CPDA-1 solution can be preserved for 35 days for transfusion. Most blood bank follow the ‘first in first out’ rule to maximize the utilization of this valuable resource. But confusion remains amongst physician and blood recipients regarding transfusion of older blood unit which can lead to difficulty in maintenance of blood storage in future. This review attempts to provide a comprehensive view of the literature about fresher and older stored red cell and their clinical effect in recipients.

**Storage lesions**

Each red blood cell loses their nuclei and organelles and thus lose the capability of synthesis new protein during maturation to make more space for haemoglobin. Haemoglobin occupies 95% of RBC volume. The ferrous iron of a haemoglobin tetramer binds with one molecule of oxygen thus making the red blood cell a highly specialized carrier of oxygen in the body. The high concentration of iron and oxygen in an RBC can exert profound oxidative stress. To mitigate it, mature RBC lose their mitochondria so that there is no oxygen consumption and the oxidized (ferric state +III) haemoglobin (i.e., methaemoglobin) is reduced back to ferrous state (+II) by methaemoglobin reductase.

In blood centres of Bangladesh, whole blood and the red cell concentrate is stored in 2⁰-6⁰C (4±2⁰C), up to 5 weeks when collected in CPDA-1 bag. During storage in this hypothermic state the chemical reactions in the red cells proceed, though at a slower rate, leading to accumulation of metabolic waste and further acidification of the medium which causes chemical and mechanical stress with no physiological countermeasures. Inevitably there are some physical and biochemical changes occur over time to the blood cells and plasma which are summarized in Table I.

<table>
<thead>
<tr>
<th>Component of blood</th>
<th>Changes while stored at 2⁰-6⁰C</th>
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<tbody>
<tr>
<td><strong>Red Blood Cell</strong></td>
<td>RBC membrane damage</td>
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<td>Shape change</td>
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<td>Cytoskeleton damage and reduce</td>
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<td>deformability</td>
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<td>ATP depletion</td>
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<td>2,3-DPG Depletion</td>
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<td></td>
<td>Haemolysis</td>
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<tr>
<td><strong>Leukocyte</strong></td>
<td>Loss of motility</td>
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<td></td>
<td>Decrease phagocytic ability</td>
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<td></td>
<td>Marked decrease of viable cell</td>
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<tr>
<td><strong>Platelet</strong></td>
<td>Loss of discoid shape</td>
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<tr>
<td></td>
<td>Apoptosis</td>
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<tr>
<td><strong>Plasma</strong></td>
<td>Decrease pH</td>
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<td></td>
<td>Decrease glucose and bicarbonate</td>
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<tr>
<td></td>
<td>Decrease sodium</td>
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<td></td>
<td>Increase potassium, lactate and ammonia</td>
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<td></td>
<td>Increase Haemoglobin</td>
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<td></td>
<td>Increase cytokines: IL-8, TNF α</td>
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<td>Increase microparticles</td>
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</table>

Altogether, these changes are termed as storage lesion. Among the components, the effect of storage on red cell is extensively studied as it is still the most used component. Red blood cell goes through several physiological and biochemical changes which are discussed below.
Oxidative damage to red blood cell

Lipid oxidation

Malondialdehyde is a product of lipid peroxidation and have been used as a marker of oxidative injury to polyunsaturated fatty acid over the last four decades.5 There are several studies that point out the increased MDA level in both red cell and plasma of stored blood both in SAGM and CPDA.6 Moreover, there is a direct relation between lipid oxidation and increased plasma haemoglobin which is a marker of membrane damage.6 Eze et al showed that with CPDA-1 preservation, changes of plasma haemoglobin are significant from day 15th to 35th, but not so in the first 15 day indicating that lipid oxidation may occur at accelerated rate from 15 days onward.7 There is not only oxidative damage to various components of red cell but also a significant reduction of total antioxidant status & catalase, glutathione peroxidase (from 14 days onward), and superoxide dismutase occur in stored blood.8

Protein oxidation

Protein carbonylation is a marker of oxidative stress which increase throughout storage and the maximum increase occur from 14-21 days.9 Al-Thani et al showed that storage induced oxidation occur in 14 different protein and important among them are spectrin alpha chain, Band 3 ankyrin-1 glyceraldehyde-3-phosphate dehydrogenase.10 Among them spectrin oxidation correlate with membrane vesiculation in stored RBC.11 Changes in the RBC membrane alongside vesiculation are, decrease interaction between spectrin-actin and fragmentation. Some authors suggest that oxidative stress may be a cause of RBC ageing.12,13

Haemoglobin oxidation

During storage there is increased methaemoglobin due to oxidation of haemoglobin and reduced level of reductase enzyme.14 Methaemoglobin and higher dissolved oxygen (increased solubility of oxygen in low temperature) form hemichromes which is non-functional and cannot bind oxygen leading to impaired respiratory function.15 Accumulation of hemichrome bind to band 3 protein. Clustering of haemoglobin causes clustering of band 3 which increase antibody binding and removal of RBC from circulation following transfusion.16

Changes in RBC morphology and its effect

Progressive changes of RBC morphology due to vesiculation and membrane loss lead to initially reversible echinocyte/stomatocyte like change to later an irreversible spherocyte which mimic hereditary spherocytosis, and also microerythrocyte 61% of which are cleared within 70 minutes of transfusion.17,18 Vesiculation and membrane loss which started from second week of storage are also responsible for decrease of membrane deformability.19 As the deformability of RBC decreases over time during storage, the rigid and fragile RBCs are removed from circulation in the first hours after transfusion and a significant portion do not survive the first 24 hour.20,21 There is also evidence that the percentage of post transfusion red cell recovery decreased with increase storage time.22

pH of stored blood and its effect

Lactate is the product of the anaerobic glycolysis that occurs in RBC and its level increases significantly over time.23 The declination of the pH level is directly proportional to increasing lactate level and Wilson et al showed that the declination is higher from day 14.24,25 Some authors suggest that lower pH is a contribution factor for haemolysis of red cells. It is not clear how lower pH contribute to storage lesion but Chang et al showed that compared to acidic AS3 solution when pRBCs stored in alkaline solution, there is significant improve in RBC quality in terms of haemolysis, phosphatidylserine externalization and microparticle production.26
Haemolysis of stored red cell

As understandably, haemolysis of stored RBCs is inevitable toward their end of storage time. Several authors reported that the haemolysis is below the expected 0.8%-1% at the end of storage time and it may vary between donor unit. Hess et al. showed that there is 30% increase in haemolysis when the storage time increased from 35 days to 42 days with additive solution and leukoreduction reduces haemolysis by 53%. Haemolysis result in releasing of iron in the blood which can directly and indirectly enhance the virulence of blood stream pathogen. And also, a retrospective analysis showed a higher prevalence of infection in patients receiving red cells 35 days or older compared to patients receiving red cells 21 days or fresher. On the other hand, Rapido et al. reported that, with normal hepatic, splenic, and renal function and intact antioxidant defences, no adverse event was observed due to non-transferrin bound iron derived from 42 days old blood transfusion in healthy volunteer.

ATP and 2,3 DPG

There is extensive discussion in literature regarding declination of ATP and 2,3-DPG during storage and their restoration after transfusion is mainly because of their role in maintaining ion pump. There is virtually no 2,3-DPG after 14 days of storage and declination of ATP is most profound after 5 weeks of storage and it begin to rise within hours of transfusion the full restoration of Na+/k+ pump activity may require 1-4 days. Heaton et al. showed that after transfusion, an average of 95% of the recipients’ pre- transfusion 2,3-DPG level was reached by 72 hours.

Electrolytes of stored Blood

Potassium which is predominantly an intracellular cation is released to extracellular fluid following cell death causes increase in ECF potassium concentration. During storage period of whole blood and red cell the potassium level increases on an average 0.5-1 mmol/l per day of refrigeration and may reach above 25 mmol/l during a 21-35 day of storage. On the contrary sodium level tends to decrease over storage duration at a rate of 4.6 mmol/l/week and can be as low as 130 mmol/l after 28 days of storage. The high level of potassium in blood that is stored for longer duration may be associated with hyperkalaemia and may have deleterious effect. And the patient who are reported to suffer most, are paediatric patients and when associated with rapid transfusion, acidosis, low cardiac output, hypocalcaemia, hypothermia, and when administered in large volume. Antwi-Baffour reported no significant changes post transfusion potassium level or related adverse event and relate it to insufficient volume transfusion. In a prospective study conducted in paediatric intensive care unit on 28 children showed no significant raise in potassium level or related adverse event following transfusion of 54 packed red blood cell (mean volume 11.8 +/- 2.8 ml/kg) with measured packed red cell potassium level as high as >25 mmol/l.

Tissue oxygenation

The sole purpose of red cell concentrate transfusion is to increase tissue oxygenation. There is interest in recent time about RBCs’ role in vasodilation. Some authors suggest the regional hypoxic vasodilation by RBCs is mediated by s-nitrosohaemoglobin and this property of RBCs is compromised due to storage. Moreover, there are evidence that the stored RBCs adhere to capillary wall more than fresher RBCs. Whether these finding have any effect on oxygenation of the hypoxic tissue is not clear though. Risbano et al. described that autologous transfusion of 42-day old blood causes decrease flow of blood in forearm. On the other hand, it was reported by Roberson et al. that there is no difference between 7 day and 42 days old blood
over oxygenation and microcirculation in tissue or brain.\textsuperscript{47} Similar result was found in a randomized controlled trial conducted on cardiac surgery patient where there was no difference in \textless 10 days and \textgreater 21 days blood over oxygenation and microcirculation.\textsuperscript{48} Walsh et al showed that storage time have no influence in regional or global tissue oxygenation in anaemic patient who are critically ill.\textsuperscript{49} Recently a randomized controlled trial evaluated the oxygen delivery ability of 7, 28 and 42 days old autologous blood. The author found no superiority of fresher unit in terms of oxygen delivery compared to older unit.\textsuperscript{50}

**Post transfusion haemoglobin level**

Physicians expect to rise haemoglobin at a rate of 1 gm/dl/unit of packed red cell. But there are several factors that influence post transfusion rising of haemoglobin. Roubinian et al described blood donor characteristics, collection and modification methods and recipient factors on post transfusion haemoglobin increment.\textsuperscript{51} Few authors showed association between age of the transfusion unit and the resultant increment of haemoglobin. Pieracci et al in a retrospective study showed that the increase of haematocrit is inversely proportional to the age of the unit transfused and significant rise is observed with less than 8-day old unit compared to the unit that is more than 35-days old.\textsuperscript{52} Another retrospective study which included patient underwent major abdominal surgery described similar result. This study showed that the mean rise of haemoglobin concentration was 0.39\textsuperscript{[0.07-0.71]} g/dl with packed red blood cell stored more than 30 days whereas it was 0.42\textsuperscript{[0.42-1.21]} g/dl with red cell unit less than 12 days old.\textsuperscript{53}

**Clinical outcome of fresher and older blood transfusion**

Whatever the storage lesions are, from clinicians and patient’s perspective it is imperative to know whether the older blood unit pose any risk to the recipients. And from the point of blood bank view, it is important to know whether the ‘first in first out’ policy should be changed or not. Several authors showed association between older blood transfusion and multiorgan failure, infection risk and death.\textsuperscript{54-56} But as most of these studies were observational, several randomized controlled trials conducted by different researchers in recent years. A meta-analysis of these trials, of which 4 were multicentre and two were single centre, was done by Zhou et al.\textsuperscript{57} The primary end point of this meta-analysis was short term mortality and secondary endpoints were duration of intensive care unit and hospital stay. The author found that fresher packed red cell (4-13 days) was not associated with decrease mortality, ICU or hospital stay compared to their older (22-36 days) counterpart. Another systematic review with meta-analysis and Trial Sequence Analysis was conducted by Rygard et al.\textsuperscript{58} Seven randomized controlled trial, six observational study and few other publications were included. They observed no effect of fresher versus older blood on death, adverse events, or post transfusion infection, and the Trial Sequence Analysis confirmed the result. Similar result was reported by Shah et al in their Chocrane Database systematic review where 22 trials were reviewed.\textsuperscript{59} The author concluded that the length of storage time of packed red cell appears to have no effect on mortality in adults. Nevertheless, when stored blood is used for massive transfusion, it may have adverse outcome compared to fresher unit.\textsuperscript{50}

**Conclusion**

It is undeniable that irreversible changes occur in red blood cells over long storage period. Though recent high-quality studies found no superiority of fresher packed red cell over older unit in adult patient, we feel that more probing is needed to ensure safety of older unit in neonate and children patient. Additionally, as microbiological tests at dispensing blood products are not routinely done in our country, infection risks of older blood unit

DOI: https://doi.org/10.37545/haematoljbd202295
should be investigated more thoroughly especially in country like Bangladesh. It should be also assessed whether certain patient can be more benefitted with transfusion of fresher unit in the context of raising haemoglobin level. For the time being, first in first out rule can be safely practised in concerned blood banks.

References


