Effect of blood storage on certain hematological parameters

Ahmed Y. Dallal Bashi*, Bashar M. Saleh**

* Dept. of Medical Biochemistry, College of Medicine, Mosul University
** Dept. of Clinical Biochemistry- Mosul Central Blood Bank, Nineveh Health General Office, Mosul.

Abstract

When blood was stored outside the body some hematological and biochemical changes will take place resulting in reduced red blood cells survival which is important drawback when transfused into the circulation of a recipient. This study was done to determine certain hematochemical effects on blood when stored during different periods of time (at 7 storage periods (from zero time up to 35 days) in both sexes using CPDA1 solution as preservative. Fifty blood donors (25 males and 25 females) who were attending the Central Blood Bank, Al-Zahrawi Hospital, Mosul (IRAQ) during the period from 1st October 2002 to 31st March 2003. A blood sample consisted of 50 ml was taken from each blood bag and this was divided into 7 portions, each contained about 7 ml of blood added into plain tubes. Blood in one of these tubes was analyzed immediately. The other six tubes were analyzed later on at intervals of 3 days, 1, 2, 3, 4, and 5 weeks. The blood samples were analyzed for hemoglobin (Hb), plasma (Hb), packed cell volume percentage (PCV %), methemoglobin and sulfhemoglobin. The results of this study showed that there was a significant decrease (P<0.05) in Hb, packed cell volume, methemoglobin and sulfhemoglobin while there was a significant increase (P<0.05) in plasma Hb. Moreover, when the Hematochemical parameters of donor blood samples of males is compared with that of female donors’ blood sample at the same periods of storage no significant differences were noted between them. The possible effects of stored blood that it may undergoes unavoidable hematochemical changes that lead to decrease active desirable substances such as hemoglobin and viable red blood cells. So blood transfusion is preferable during less than 7 days of storage. Moreover the Hematochemical parameters of donor blood samples of males was compared with that of female donors blood sample at the same periods of storage and no significant differences was noted between them.

Key Word: hemoglobin (Hb), plasma (Hb), packed cell volume percentage, methemoglobin and sulfhemoglobin.

Introduction

Blood has always considered as essential for the maintenance of life. The ancients undoubtedly have the experience of seeing life literally flow out of the body from hunting wands (1). Villans recorded the earliest administration of blood I.V. of human in 1492 where the blood was transfused to pope innocent VIII; unfortunately, he had suffered a stroke and died (2).

Later many unsuccessful blood transfusion was done (3) till the year 1818, where the revival of blood transfusion was attributed by Blundell an English obstetrician where he devised the prototype of the modern syringe and receptacles for transferring blood from donors to recipients. Many trials were done until the year 1914 at which the beginning of the modern era of blood transfusion technique (4). Sodium citrate was the first anticoagulant used (5). Rous and Turner in 1916 indicated that when glucose added to citrate it would improve the preservation of citrate (4). On that, the first blood bank was established and opened in Chicago in 1937. Later on, Molison in 1943 improved blood transfusion by adding acid citrate dextrose anticoagulant (ACD) mixed with preservative solution that made the...
storage of whole blood possible up to 21 days\(^6\). Gibson introduced a new improved preservative solution in 1957 containing citrate, phosphate, dextrose (CPD) that increases the storage age up to 28 days\(^7\). This storage solution was modified in USA in 1978 by incorporating adenine to citrate-phosphate-dextrose and called CPDA1, which is, regarded the latest blood preservative. It increases blood storage time up to 35 days and it is nowadays in every blood bank in the world\(^8\).

The goal of blood preservation is to provide viable and functional blood components for patients needing blood transfusion\(^8\). Viability is a measure of in vivo red blood cells survival following blood transfusion\(^8\). Decreased RBC viability is correlated with lesion of storage that is associated with various biochemical changes including decreased pH, a build up of lactic acid, a decrease in glucose consumption, a decrease in adenosine triphosphate levels and a loss of RBC functions\(^9\).

Rossmussen 1961 found that there is some loss of the erythrocyte during storage when stored in ACD solution. He found that the post transfusion survival virtually is 100% then this decline by slightly more than 1% per day to reach about 90% in about one week of storage and about 70% in three weeks of storage. This means that Hb concentration remains declining by increased period of storage\(^10\).

Approximately all of the Hb in the blood contained with the RBC and only minute amount of less than 0.025 g/L of Hb normally is released into the plasma due to destruction of erythrocyte Hb. It is believed that it may occurs as a result of transfusion reaction\(^11, 12\).

Gibson 1957 described as a storage lesion of cells during storage by refrigerator that may contribute to increase destruction of red cells leading to decrease Hb level and increase of plasma Hb several folds above normal level\(^13\).

The red cell volume is maintained and regulated by the bone marrow, which under steady state condition is accurately replace the red cell loss\(^13\).

The haematocrit is the simplest and most widely used test by which it is possible to estimate the size of the red cell volume. In most anemic patients, it gives an excellent approximation of the total red cell volume and functional estimation of the oxygen carrying capacity and bulk viscosity of the blood.

Methemoglobin is defined as an oxidation product of hemoglobin. Each hemoglobin molecule contains one iron atom in a reduced form hemoglobin which exist as ferrous, and legend to oxygen\(^14\). Methemoglobin formation occurs when the ferrous is oxidized to ferric and it is incapable of binding legend\(^15\).

When hemoglobin value decreases during storage because of hemolysis of RBC then Methemoglobin and sulfhemoglobin values are similarly reduced\(^16\). Sulfhemoglobin is a bright green dichromic haem protein compound occurring in vivo in the RBC in the condition known Sulfhemoglobinemia which develop as a consequence of interaction of many forms of hemoglobin with chemicals such as hydrogen disulfide\(^17\).

Once Sulfhemoglobin formed, it cannot be reconverted to hemoglobin it can bind with carbon monoxide but not with oxygen. The only mean of removal of Sulfhemoglobin is erythrocytes destruction as happens during blood storage\(^18\).

The aim of this work is to study the effects of storage on blood in both sexes using CPDA1 solution as preservative by measuring several hematociological parameters at 7 storage periods.
Materials and Methods

Fifty (50) subjects were shared who attended to Mosul Blood Bank, Al-Zahrawi Hospital, Mosul (IRAQ) as blood donors from 1st October 2002 to 31st March 2003 (25 adult males and 25 adult females). Male's ages were ranged between 20-45 years with a mean of 31.2 years while the female's ages were ranged between 21-42 years with a mean of 30.6 years. All subjects were serologically examined for hepatitis B virus, hepatitis C virus and HIV before blood was donated.

The recommended quantity of blood (437 ml) was obtained from all donors in this study by antecubital venepuncture, added to special blood bags containing 63 ml of CPDA1 anticoagulant solution and given to the blood bank to be stored. A blood sample consisted of 50 ml from each blood bag was taken. Each sample was divided into 7 portions, each portion consisted of 7 ml of blood was added into plain test tube. One of these tubes was analyzed immediately, which was regarded as control or at zero time. The other six tubes were kept in the blood bank refrigerator at 2-8°C to be analyzed later on at 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, and 5 weeks intervals. Each sample was analyzed hematochemically for hemoglobin, plasma hemoglobin, Methemoglobin, Sulfhemoglobin, and packed cell volume percentage (PCV%).

All reagents used through this study, and all the analysis were obtained and performed from and at the Clinical Chemistry Laboratory in the Department of Biochemistry, Mosul College of Medicine, University of Mosul.

1-Hemoglobin was estimated by cyanmethemoglobin method, which is a specific method for estimation of hemoglobin (19).

2. Plasma hemoglobin:
Plasma hemoglobin was estimated by benzidine-haem color reaction (20).

3. PCV%:
Packed cell volume percentage was estimated by micro capillary tube method (21).

Methemoglobin and Sulfhemoglobin:
The method followed was a spectrophotometric measurement of methemoglobin and sulfhemoglobin as described by Fair banks (19).

Statistical analysis: The following methods were used for the analysis of data (22):

1. Standard statistical methods were used to determine the mean, and standard deviation.
2. Dunnet t-test was used to find the effect of blood storage on its hematochemical parameters. The blood at zero time is considered as a control.
3. All values quoted as the mean +/- SD. The difference between observations were considered significant at P <0.05.
4. Correlation analysis was used to find the relationship between hematochemical parameters and periods of storage.

Results

Hemoglobin levels in both males and females showed no significant changes during the first 3 days of storage when compared with blood at zero time (Tab 1 & 2). Then at day 7 it started to decrease significantly (P<0.05) in males and females (Tab 1 & 2), with about 5% decrease in males and 6% in females. Both continued to decrease gradually and more significantly on days 14, 21, 28, and 35 of storage 10%, 13%, 16% and 20% decrease respectively in males (P<0.01), and 13%, 15%, 18% and 21% decrease respectively in females (P<0.01).

Concerning plasma hemoglobin, it showed no significant increase during the first three days of storage (P > 0.05). Then at day, 7 of storage plasma...
The hemoglobin concentration started to increase significantly (p < 0.05) in males and females (Table 1 & 2) with 32% increase in males and 27% increase in females. Both continued to increase gradually and more significantly on days 14, 21, 28, and 35 of storage. There were 48%, 66%, 96% and 130% increase respectively in males (p < 0.01) and 50%, 80%, 120% and 148% increase respectively in females (p < 0.01).

The packed cell volume behaves like that of hemoglobin during storage (Table 1 & 2 and Fig. 3 & 4). The packed cell volume decrease was not significant for the first 3 days of storage in males and females (p > 0.05).

At day 7 it started to decrease significantly in males and females (p < 0.05). The decrease in packed cell volume continued during storage and become more significant in both males and females (p < 0.01) (Table 1 & 2).

The normal concentration of Methemoglobin in blood is less than 2.5 g/L (23). The methemoglobin level showed no significant change during the first seven days of storage (P>0.05). This can be due to that blood is still fresh and stable and hemolysis is not clear. After 14 days of storage methemoglobin starts to decrease significantly (p< 0.05) in both males and females (Table 1 & 2) with 16% decrease in males and 20% decrease in females. It continued to decrease gradually and more significantly (p< 0.01) on days 21, 28, and 35 of storage. The decreases were 20%, 23% and 26% in males respectively and 16%, 19% and 33% in females respectively.

Sulfhemoglobin showed significant decrease starting after 7 days of storage in males (p< 0.05), while in females it showed significant decrease starting at day 14 of storage (Table 1 & 2). Both continued to decrease gradually and more significantly on day 21, 28, and 35 of storage.

### Discussion

When blood was stored outside the body some hematological and biochemical changes will takes place resulting in reduced red blood cells survival which is important drawback when transfused into the circulation of a recipient (6).

Hemoglobin levels in both males and females showed no significant changes during the first 3 days of storage when compared with blood at zero time (Tab 1 & 2), probably because the blood was still fresh. Then at day 7 it started to decrease significantly (P<0.05) in males and females (Tab 1 & 2) which can be accounted to the hemolysis that occurs during storage. This result is in agreement with the results of Donahue et al., (24). The erythrocyte hemolysis can be attributed to several causes including: old erythrocytes age hemolysis (ranged between 100-120 days) (25,26), improper storage of blood (higher than 8°C in blood bank refrigerator) (27), or blood bags not mixed periodically leading to decreased 2, 3-diphosphoglycerate which is very important to preserve RBC and maintains physiological functions (28). However, although hemoglobin decrease was significant during storage however, its concentration in the blood was still within the acceptable normal range value.

Concerning plasma hemoglobin, the results showed a significant increase of the plasma hemoglobin (Table 1 & 2 and Fig. 1 & 2) which might be due to that hemoglobin being released from the red blood cells into the plasma due to hemolysis during storage. Therefore, the level of plasma hemoglobin was increased gradually as the period of storage was increased. These results are in agreement with the results of Donahue et al., (24) and these results can be attributed to hemolysis of blood during storage.

The packed cell volume behaves like that of hemoglobin during storage (Table 1 & 2 and Fig. 3 & 4) as the
measurement of packed cell volume was an index for real hemoglobin. The packed cell volume decrease was not significant for the first 3 days of storage in males and females ($p > 0.05$) as the blood was still fresh and hemolysis was not clear. At day 7 it started to decrease significantly in males and females ($p < 0.05$). The decrease in packed cell volume continued during storage and become more significant in both males and females ($p < 0.01$) (Table 1 & 2). These results were in agreement with results of Bensinger et al. $^{(29)}$. The cause of decrease in packed cell volume can be attributed to erythrocytes hemolysis.

The normal concentration of Methemoglobin in blood is less than 2.5 g/L$^{(23)}$. The methemoglobin level showed no significant change during the first seven days of storage ($P > 0.05$). This can be due to that blood is still fresh and stable and hemolysis is not clear. After 14 days of storage methemoglobin started to decrease significantly ($p < 0.05$) in both males and females (Table 1 & 2). It continued to decrease gradually and more significantly ($p < 0.01$) on days 21, 28, and 35 of storage. The methemoglobin behaves similar to hemoglobin during blood storage as methemoglobin is one of hemoglobin derivatives. It undergoes the same degree of hemolysis as hemoglobin during storage. These results were in agreement with the results of Finch et al. $^{(30)}$.

As a conclusion:

1. There is a significant decrease in hemoglobin concentration and packed cell volume and significant increase of plasma hemoglobin after one week of storage. These changes continued by advancing periods of storage. The methemoglobin & sulfhemoglobin decrease after 2 weeks of storage. However, this decrease will make no difference because both are not desirable substances as both represent non-active hemoglobin.

Therefore, it is better to give patients fresh blood during less than 7 days of storage in order to decrease the levels of non-viable red blood cells.

2. The possible effects of stored blood on the recipient health can be summarized by that all stored blood undergoes unavoidable hematochemical changes that lead to decrease active desirable substances such as hemoglobin and viable red blood cells. So blood transfusion is preferable during less than 7 days of storage.

3. When the Hematochemical parameters of donor blood samples of males is compared with that of female donors blood sample at the same periods of storage no significant differences was noted between them.
Effect of blood storage on certain hematological parameters

References


3. Hoff HE. The first blood transfusion French or English?. J His Med 1963; 18: 360-84.


Effect of blood storage on certain hematological parameters


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Storage Time</th>
<th>Hb (g/L)</th>
<th>PCV (L/L)</th>
<th>Plasma Hb (g/L)</th>
<th>MetHb (g/L)</th>
<th>SulfHb (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>146 ± 3.8</td>
<td>0.438 ± 0.1</td>
<td>0.012 ± 0.002</td>
<td>0.78 ± 0.16</td>
<td>0.65 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td>144 ± 3.9</td>
<td>0.434 ± 0.11</td>
<td>0.013 ± 0.002</td>
<td>0.75 ± 0.16</td>
<td>0.60 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>139 ± 4.4</td>
<td>0.418 ± 0.12*</td>
<td>0.016 ± 0.002*</td>
<td>0.71 ± 0.15*</td>
<td>0.56 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>133 ± 4.6</td>
<td>0.401 ± 0.13*</td>
<td>0.017 ± 0.002*</td>
<td>0.66 ± 0.16*</td>
<td>0.52 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>128 ± 5.0*</td>
<td>0.390 ± 0.13*</td>
<td>0.02 ± 0.002*</td>
<td>0.63 ± 0.15*</td>
<td>0.48 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>124 ± 5.0*</td>
<td>0.381 ± 0.13*</td>
<td>0.023 ± 0.002*</td>
<td>0.60 ± 0.15*</td>
<td>0.47 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>5 weeks</td>
<td>120 ± 5.0*</td>
<td>0.367 ± 0.26*</td>
<td>0.027 ± 0.002*</td>
<td>0.57 ± 0.15*</td>
<td>0.44 ± 0.10*</td>
</tr>
</tbody>
</table>

*Significant difference from 0 times according to Dunnet test in each column.

**Table (2)** Hematochemical changes during blood storage for females.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Storage Time</th>
<th>Hb (g/L)</th>
<th>PCV (L/L)</th>
<th>Plasma Hb (g/L)</th>
<th>MetHb (g/L)</th>
<th>SulfHb (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>136 ± 4.0</td>
<td>0.409 ± 0.1</td>
<td>0.011 ± 0.002</td>
<td>0.75 ± 0.16</td>
<td>0.63 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
<td>134 ± 3.9</td>
<td>0.404 ± 0.11</td>
<td>0.012 ± 0.002</td>
<td>0.73 ± 0.17</td>
<td>0.61 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>1 week</td>
<td>129 ± 4.4*</td>
<td>0.390 ± 0.10*</td>
<td>0.014 ± 0.002*</td>
<td>0.63 ± 0.17</td>
<td>0.57 ± 0.45*</td>
</tr>
<tr>
<td></td>
<td>2 weeks</td>
<td>122 ± 45*</td>
<td>0.377 ± 11*</td>
<td>0.016 ± 0.002*</td>
<td>0.60 ± 0.17*</td>
<td>0.54 ± 0.95*</td>
</tr>
<tr>
<td></td>
<td>3 weeks</td>
<td>118 ± 5.0*</td>
<td>0.367 ± 0.11*</td>
<td>0.019 ± 0.002*</td>
<td>0.56 ± 0.18*</td>
<td>0.49 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>4 weeks</td>
<td>114 ± 5.0*</td>
<td>0.357 ± 0.11*</td>
<td>0.024 ± 0.002*</td>
<td>0.53 ± 0.18*</td>
<td>0.71 ± 0.10*</td>
</tr>
<tr>
<td></td>
<td>5 weeks</td>
<td>110 ± 5.0*</td>
<td>0.346 ± 0.11*</td>
<td>0.027 ± 0.002</td>
<td>0.50 ± 0.17*</td>
<td>0.44 ± 0.09*</td>
</tr>
</tbody>
</table>

*Significant difference from 0 time according to Dunnet test in each column

![Figure (1)](image-url)

*Significant difference from 0 time according to Dunnet test in each column

**Figure (1)** The variation in plasma hemoglobin concentration with storage for males.
Effect of blood storage on certain hematological parameters

Figure (2) The variation in plasma hemoglobin concentration with storage for females.

Figure (3) The variation in PCV with storage for males.
Effect of blood storage on certain hematological parameters

Figure (4) The variation in PCV with storage for females.

* Significant difference from 0 time according to Dunnet test in each column