

**Serial changes in whole blood units during storage to evaluate safety for blood transfusion**

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**ABSTRACT**

During storage of whole blood units in blood banks, certain changes are expected like functional and biochemical changes. This work aims to evaluate safety of whole blood units for blood transfusion. In this study thirty units of whole blood were selected for study. Each unit was assessed serially at days 1, 7, 14, 21 and 28 of storage by visual assessment, plasma potassium level, plasma glucose level and plasma Hb (free Hb). The maximum acceptable percentage of hemolysis was considered to be 0.8%.

The results indicated that visual assessment of whole blood units showed evidence of pinkish discoloration of supernatant plasma in 14 out of 30 units (i.e 46.67%) at days 7, 14, 21 and 28 of storage. There was a significant rise in plasma potassium concentration ( $P < 0.05$ ) in 100% of units from day 1 to 28 of storage while there was a significant fall in plasma glucose level ( $p < 0.05$ ) in 100% of units from day 1 to day 28 of storage. Free Hb levels were significantly elevated from day 1 to 28 of storage reaching a maximum value of 100 mg/dl (i.e 0.36% hemolysis which is much lower than the maximum percentage of acceptable hemolysis).

In conclusion, serial changes of whole blood units depending on both visual and laboratory assessment, but not on visual assessment alone, were within the acceptable limits of safety for blood transfusion.

**Key words:** Whole blood units, Serial changes, hemolysis.

**INTRODUCTION**

Stored whole blood units in blood banks are exposed to low temperature ( $4^{\circ}\text{C}$ ) while the optimal temperature for red cell function is  $37^{\circ}\text{C}$ , so that functional, biochemical and membrane structural changes are expected to occur during storage like hemolysis, decrease in ATP production and increase in plasma potassium level<sup>1</sup>. Anticoagulants and additives that are added to whole blood units are developing continuously through several years to ensure prolonged red cell survival as much as possible through the availability of glucose for energy, citrate for anticoagulation and others like phosphate, adenine, mannitol, and saline for prolonged and best survival of red blood cells before and after blood transfusion<sup>2</sup>. Heparin, as anticoagulant, was used during the second world war, after that, ACD (Acid Citrate Dextrose) was used as anticoagulant and preservative solution to keep whole blood units for up to 21 days. CPD (Citrate Phosphate Dextrose) has again similar survival period for up to 21 days,

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while with the addition of adenine (CPDA)(Citrate Phosphate Dextrose Adenine), survival of red cells had been prolonged for up to 35 days. The introduction of SAGM (Saline Adenine Glucose Mannitol), and ADSOL(Adenine Dextrose solution) had prolonged red cell survival for up to 42 days during storage<sup>3</sup>. Whole blood units are mainly spared for blood transfusion to correct anemia when there is a medical indication for blood transfusion. Packed red cells are preferable to correct anemia when there is a medical indication for blood transfusion<sup>4</sup>. The loss of membrane function is most likely related to some abnormality in maintenance of cytoskeleton of red cells. These changes appear to occur independent of ATP levels<sup>5</sup>. Red cell hemolysis, as a major challenge during storage, can be assessed by spectrophotometric method, photometric method, and microplate method<sup>6&7</sup>.

## MATERIALS AND METHODS

A total number of 30 whole blood units (with CPDA<sub>1</sub> anticoagulant and preservative) stored in Karbala blood bank were included in the study. The study was done in a legal private laboratory for specific hematological investigations (Zaid Bin Ali private laboratory) in Iraq, Karbala city, Al Mualimeen sector. Serial assessment of whole blood units at days 1,7,14, and 28 of storage was done by doing visual assessment of whole blood units for pinkish discoloration of supernatant plasma without mixing of blood. Blood samples were taken from the available segments of stored blood bags for serial assessment of plasma potassium level (using Human German company kit by doing photometric assay at 578 nm against reagent blank), plasma glucose level(using Randox glucose United Kingdom company kit by doing spectrophotometric assay at 500 nm), and plasma hemoglobin level i.e free hemoglobin level using TetraMethyl Benzidine method (TMB) measuring absorbance at 600 nm spectrophotometrically against deionized water.

Calculation of percentage of hemolysis was done as follows:

$$(100-\text{pcv}) \times \text{plasma Hb}(\text{g}\%) / \text{total Hb}(\text{g}\%)$$

Paired T-test was used to compare between study results. P value of < 0.05 was considered to be statistically significant using Pearsons correlation coefficient.

## RESULTS

Visual assessment of whole blood units showed evidence of pinkish discoloration of supernatant plasma in 14 out of 30 units (i.e 46.67%) at days 7, 14, 21 and 28 of storage (2 at day 7, additional 2 at day 14, additional 4 at day 21 and additional 6 at day 28); however, no one showed significant hemolysis as reflected by free plasma hemoglobin level. As shown in table 1, there was a significant rise in plasma potassium concentration (P<0.05) in 100% of units from day 1 to 28 of storage while there was a significant fall in plasma glucose level (p<0.05) in 100% of units from day 1 to day 28 of storage. Free Hb levels were significantly elevated from day 1 to 28 of storage reaching a maximum value of 100 mg/dl (i.e 0.36% hemolysis on day 21 of storage), and maximum value of 152.2 mg/dl (0.54% hemolysis) on day 28 of storage which are lower than 0.8% ( the maximum percentage of acceptable hemolysis).

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**Table 1. Serial assessment of plasma potassium and glucose levels (mean  $\pm$  2SD)**

		Days of storage				
		1	7	14	21	28
1.	Mean plasma K <sup>+</sup> mmol/L	4.03 $\pm$ 0.5	8.7 $\pm$ 1.9	14.8 $\pm$ 3.1	20.9 $\pm$ 4.6	25.1 $\pm$ 6.0
2.	Mean plasma glucose mg/dl.	359.3 $\pm$ 39.3	267.8 $\pm$ 41.2	214.3 $\pm$ 46.4	169.2 $\pm$ 51.4	140.8 $\pm$ 56.1

**Table 2. Serial measurements of plasma hemoglobin and percentage of hemolysis.**

		Days of storage				
		1	7	14	21	28
1.	Mean plasma Hb (g%).	25.7 $\pm$ 10.6 15.1-36.3	49.5 $\pm$ 20.1 29.4-69.6	77.8 $\pm$ 22.2 55.6-100	108.8 $\pm$ 20.9 87.9-129.7	128.0 $\pm$ 24.2 103.8-152.2
2.	% of hemolysis.	0.09% 0.05-0.13	0.17% 0.1-0.25	0.28% 0.2-0.36	0.39% 0.31-0.46	0.46% 0.37-0.54

### Conclusion

Serial changes of whole blood units depending on both visual and laboratory assessment, but not on visual assessment alone, were within the acceptable limits of safety for blood transfusion with the presence of a percentage of hemolysis which is less than the maximum acceptable level of hemolysis. Visual assessment of whole blood units alone is not enough and should be confirmed by laboratory tests for suspected whole blood units before discarding such units.

### Discussion

Whole blood units are exposed to cooling, handling and processing which can lead to variable changes, including hemolysis; however, biochemical changes in stored whole blood units are a fact and such changes are increased by more handling and more processing<sup>1</sup>. Pinkish discoloration of supernatant plasma of whole blood units was seen in 14 out of 30 units (46.67%); however, no one of them showed evidence of hemolysis more than the maximum acceptable level of hemolysis (0.8%). So that visual assessment may be misleading and hemolysis, once suspected, should be confirmed by laboratory ways of assessment<sup>8</sup>. In this study, a significant rise in plasma potassium level from day 1 to day 28 of storage was found which is matching with other studies<sup>9</sup>. A significant fall in plasma glucose level is explainable since glucose is the main element for red cell metabolism through glycolytic pathway. Studies on stored red cell units showed that the concentration of glucose is significantly higher in saline adenine glucose mannitol (SAGM) containing blood units than CPDA<sub>1</sub> containing blood units, indicating prolonged survival of red cells in SAGM containing blood units through prolonged ability to generate ATP through glycolytic pathway<sup>10</sup>. This study showed that there is a significant rise in supernatant plasma hemoglobin (free hemoglobin) reaching a maximum

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value of hemolysis of 0.46% on day 21 of storage and a maximum of 0.54% on day 28 of storage which is lower than the maximum acceptable value for hemolysis (0.8%). Similar results were established in Greenwalt TJ study which showed a maximum value of hemolysis 0.49% on day 28 of storage<sup>11</sup>.

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التغيرات المتتالية للدم أثناء فترة خزنه في مصرف الدم لتقييم سلامة وأمان نقل الدم للمرضى.

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### المستخلص

أثناء فترة خزن الدم في مصرف الدم هناك عدد من التغيرات المتوقع حدوثها كالتغيرات الوظيفية للخلايا والتغيرات الكيميائية. والهدف من هذه الدراسة تقييم سلامة وأمان الدم المخزون في مصرف الدم . تمت دراسة 30 وحدة دم (30 كيس دم) في مصرف الدم بإجراء الفحوصات العينية والمختبرية في الأيام 1,7,14,21,28 من الخزن أي النظر بالعين المجردة للتغيرات في لون بلازما الدم وإجراء الفحوصات المختبرية لكل وحدة دم والتي شملت فحص البوتاسيوم في البلازما وفحص السكر في البلازما وفحص نسبة الهيموكلوبين الحر في البلازما كعلامة تشير الى تكسر خلايا الدم الحمراء. أوضحت نتائج الفحص بالعين المجردة وجود 46.67% من وحدات الدم (أكياس الدم) ملونة باللون الوردي في بلازما الدم كعلامة أولية لتكسر خلايا الدم الحمراء في الأيام 1,7,14,21,28 من الخزن. وكان هناك ارتفاع مهم إحصائياً في مستوى البوتاسيوم في البلازما من يوم 1 الى يوم 28 من الخزن في جميع وحدات الدم بينما كان هناك انخفاض مهم إحصائياً في مستوى السكر في البلازما من يوم 1 الى يوم 28 من الخزن في جميع وحدات الدم. وكان هناك ارتفاع مهم إحصائياً في مستوى الهيموغلوبين الحر في البلازما فكانت اعلى قيمة له 100 ملغ/ديسي لتر في يوم 21 من الخزن والتي تقابل نسبة تكسر خلايا الدم الحمراء بقيمة 0,36% وكانت اعلى قيمة له 152,2 ملغ /ديسي لتر في يوم 28 من الخزن وتقابل نسبة تكسر خلايا الدم الحمراء بقيمة 0,54% وهي اقل من القيمة العليا المقبولة لتكسر خلايا الدم الحمراء المخزونة (0,8%). وقد امكن استنتاج ان التغيرات المتتالية لوحدات الدم المخزون في مصرف الدم اعتماداً على الفحوصات العينية والمختبرية معا وليس اعتماداً على الفحوصات العينية فقط تؤكد سلامة وأمان وجاهزية الدم المخزون لعملية نقل الدم للمرضى.