ORIGINAL ARTICLE

HAEMATOLOGICAL AND BIOCHEMICAL EFFECTS OF TRANSFUSION OF STORED BLOOD IN TRANSFUSION-DEPENDENT THALASSEMIA PATIENTS

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Background: Thalassemia is a genetic disease in which there is an imbalance in the synthesis of globin polypeptide chains. The current study aimed to determine the haematological and biochemical effects of transfusion of seven days stored blood in transfusion-dependent thalassemia patients. **Methods:** A quasi-experimental study was conducted between January and July 2021 at Muhammad College of Medicine, Peshawar. A total of 20 transfusion-dependent thalassemia patients were selected. The impact of transfusion of 7-days old blood on haemoglobin levels, serum LDH, serum electrolytes, serum-free haemoglobin, serum bilirubin, serum-free iron, serum ferritin, and C-reactive protein were measured. Variations in pre-transfusion and post-transfusion samples were determined using paired-samples *t*-test, and $p<0.05$ was considered significant. **Results:** There was a non-significant difference in increase of haemoglobin levels ($p=0.543$) after transfusion of fresh and stored blood. Similarly, RBC counts, MCV, MCH, MCHC showed a slightly lower increase as compared to fresh blood. No differences were seen in platelet count between the two groups. However, the rise in white cells was significantly higher after transfusion of 7-days stored blood as compared to fresh blood ($p=0.002$). A non-significant increase in post-transfusion LDH ($p=0.13$), direct bilirubin ($p=0.76$) and indirect bilirubin ($p=0.45$) was seen. No differences in creatinine, glucose, and uric acid variations were found. Levels of C-reactive protein showed a significantly higher raise when 7-days stored blood was transfused in comparison with fresh blood ($p=0.012$). **Conclusion:** At least seven days stored blood can be safely transfused to transfusion-dependent thalassemia patients.

Keyword: Thalassemia, blood transfusion, banked blood, Peshawar, Pakistan

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INTRODUCTION

Red blood cell transfusion is one of the most common medical procedures performed in the world. In the United States of America, an estimated 49 numbers of blood units per 1,000 of the population are transfused in various settings each year.[1] Most blood transfusions are provided in hospital settings to patients undergoing surgery, or to those requiring multiple blood transfusions such as patients with thalassemia or aplastic anaemia.[2] To establish a continuous supply of safe blood, a comprehensive supply chain mechanism of blood collection, screening, storage, and transfusion is required.[3] Blood units are stored in blood banks in refrigerators for up to 42 days before transfusion. During storage, red cells undergo multiple physiologic changes. These generally described as 'storage lesions' include haemoglobin oxidation and release of free haemoglobin, RBC membrane structural degradation, and increase cell fragility. Metabolic abnormalities accumulate over time as well which include accumulation of lactate and depletion of ATP and 2,3-diphosphoglycerate. These cells when transfused, are at risk of haemolysis, releasing free iron causing oxidative damage to organs.[4]

Recent clinical and laboratory data suggest that increasing the duration of storage is associated with increased morbidity and mortality. A meta-analysis reported no significant differences in survival comparing transfusion of fresh blood (1–10 days old) with older stored blood (2–3 weeks) in clinical trials. However, an increased risk of death was found with increasing duration of storage in 31 observational studies.[5] Rapido *et al* showed that autologous transfusion of stored blood after 5 weeks showed marked extra vascular haemolysis, saturated serum transferrin, and circulating free transferrin.[6] Post-42 days of storage, transfusion of red cells produced extravascular haemolysis and circulating non-transferrin bound iron and was associated with proliferation of Escherichia coli.[7] In another study, up to 21 days stored blood transfusion was not found to be associated with multi-organ dysfunction in patients undergoing cardiac surgery.[8] However, the majority of these studies are either performed on healthy volunteers or clinically gross outcomes such as morbidity or mortality. Based on this lack of evidence, the American Association of Blood Banks allows transfusion of up to 42 days stored blood. Biochemical evidence of transfusion of stored blood shows that transfusion of

older red cells results in lysis and causes an inflammatory state.[9] This in an otherwise healthy individual might be of no clinical consequence, however, may be harmful when transfused to patients with compromised hemodynamics.

Thalassaemia major is characterized by severe transfusion-dependent anaemia, ineffective erythropoiesis, and extramedullary haematopoiesis. In addition, there is an enlargement of the spleen with hypersplenism and increased red cell clearance in the sinusoids.[10] Transfusion of older red cells to patients with splenomegaly may probably destroy red cells in the spleen, resulting in the release of haemoglobin, free iron and causing an inflammatory state. There is a need to assess the effects of transfusion of stored blood in such patients. The current study aimed to determine the impact of transfusion of 7-days stored blood on haematological and biochemical parameters in transfusion-dependent thalassaemia.

MATERIAL AND METHODS

This quasi-experimental study was designed to achieve the objectives. Blood samples were collected from patients enrolled at Fatimid Foundation Thalassaemia Care Centre, Peshawar, and the laboratory work was performed at Pathology Department of Muhammad Medical College, Peshawar. The study was performed from January to July, 2021 after ethical approval. The purpose of the study, procedure, risks, and benefits of the study were explained to the parents of the patients and written consent was taken. Patient demographic data such as age, sex, address, phone number, and transfusion demand per month were collected. A personal number was allocated to each patient. A total 20 transfusion-dependent thalassaemia patients with age 5 years and above were included in the study. Blood group of each patient was checked for ruling-out the chance of alloimmunization. Blood donations were collected as per standard practice in blood bags and were screened for Hepatitis B and C, HIV, and Syphilis. Blood bags were then centrifuged in a high-speed blood bank centrifuge (4,000 rpm) for plasma separation. Plasma was transferred in another blood bag and red cell concentrate was stored in the standard blood bank. Blood bags were separated into 'fresh' and were transfused within 2-days of collection, or were labelled as 'stored' and kept in the refrigerator for 7 days before transfusion.

In the first visit, each patient received a transfusion of fresh blood (2-days of storage). Prior to transfusion, a complete general physical examination was performed. Subsequently, blood samples were collected in EDTA tubes and gel tubes containing z-serum clot activator. Samples were transferred to the laboratory for analysis. After that, red cell concentrate was transfused to the patient as standard practice in the centre. 24-hours after the transfusion, the patient was examined again, and blood samples were collected in EDTA and gel tubes. The patient was informed of their next transfusion schedule after a 2–4 weeks depending upon their haemoglobin level. Upon next visit, the same examination, blood sampling, and transfusion procedure was adopted, except this time, 7-days stored blood was transfused. A 24-hour post-transfusion sample was obtained as before.

In Laboratory, complete blood count (CBC) of blood samples was calculated on Abbott's Cell-Dyn 3200 haematology analyzer. The blood sample was aspirated, and a complete blood count was obtained on the attached computer screen. The machine contains pre-installed reagents for blood count which were supplied by the company. Serum ferritin levels of the samples were measured on Roche's Cobas e411 analyzer. The blood sample was centrifuged at 4000 rpm for 3–5 minutes to obtain blood serum. A 10 µl serum was then aspirated in Roche's Cobas e411 analyzer to measure the serum ferritin level as the machine contain a pre-installed ferritin kit. The machine was pre-calibrated through the company's supplied reagent before the test. Alanine aminotransferase (ALT), bilirubin, uric acid, C-reactive protein (CRP), lactate dehydrogenase (LDH) and glucose levels of blood samples were measured on Roche's Cobas C111 analyzer. The test type was selected from the machine's software and obtained results were recorded.

Participants' demographic and laboratory parameters were entered in Microsoft Excel 365 and statistical analysis was performed through SPSS-23. Differences in each laboratory parameter were calculated. Mean differences and standard deviation were calculated. Paired sample *t*-tests were performed to compare the differences in parameters between fresh blood transfusion and stored blood transfusion, and $p<0.05$ was considered significant.

RESULTS

The study was conducted on 20 thalassemia patients including 13 (65%) female and 7 (35%) male patients with a mean age of 17.75±5.03 years. The demographic and clinical data of the patients is shown in Table-1. Transfusion of 'fresh' and 'stored' blood resulted in an increase in blood Hb levels in 24-hour post-transfusion samples. This increase was more when fresh blood was transfused (1.55 mg/dL) compared to old blood (1.35 mg/dL). However, this difference was not statistically significant ($p=0.543$). Similarly, other red cell parameters (RBC counts, MCV, MCH, MCHC) showed a slightly lower increase as compared to fresh blood but these results were not statistically significant. No differences were seen in platelet count between the two groups. Notably, an increase in white cell counts was seen in both groups. The rise in white cells was

significantly higher after transfusion of 7-days stored blood (1.01×10^9/L±2.03) as compared to fresh blood (1.82^9/L±1.1) (paired sample *t*-test, p=0.002) (Table-2).

Table-1: Demographics and clinical features of patients (n=20) [Mean±SD, n (%)]

Variables	Observed Values	
Age (Years)	17.55±5.031	
Gender Ratio (Male:Female)	1:1.7	
	Yes	No
Splenomegaly	2 (10)	18 (90)
Hepatomegaly	4 (20)	16 (80)
Jaundice	9 (45)	11 (55)
Haemoglobin (g/dL)	7.58±1.59	
Red Blood Cells (10^{12}/L)	2.86±0.64	
White Blood Cells (10^9/L)	6.25±3.34	
Platelets (10^9/L)	199.9±147.2	
Packed Cell Volume (%)	22.6±5.06	
MCV (fL)	79.5±4.85	
Mean Corpuscular Haemoglobin (pg)	26.81±2.36	
MCHC (g/dL)	33.70±1.75	
Bilirubin Direct (mg/dL)	0.53±0.30	
Bilirubin Indirect (mg/dL)	1.11±0.99	
Glucose (mg/dL)	125.3±76.77	
Uric Acid (mg/dL)	4.35±1.10	
Lactate Dehydrogenase (U/L)	345.15±173.36	
Creatinine (mg/dL)	0.31±0.10	
C-reactive protein (mg/dL)	17.30±39.57	
Ferritin (ng/mL)	2901.35±1855.63	
Iron (µg/dL)	547.19±235.86	

Table-2: Difference in haematological parameters fresh blood *vs* 7-days stored blood (Mean±SD)

Variables	Increase in post-fresh RCC transfusion	Increase in post-stored RCC transfusion	*p*
Hb	1.55±0.64	1.35±0.46	0.543
RBC	0.52±0.25	0.57±0.24	0.89
WBC	1.01±1.1	1.82±2.03	0.002*
Platelet	19.9±18.17	22.85±26.55	0.65
PCV	4.67±2.51	3.57±1.91	0.91
MCV	1.67±1.87	1.25±0.91	0.17
MCH	0.93±0.64	0.83±0.77	0.46
MCHC	1.33±1.06	0.87±0.84	0.85

*Significant

The older transfused red cells may undergo lysis resulting in increased LDH and bilirubin levels. To assess this, differences in LDH values, pre-and post-transfusion of fresh and 7-days stored blood was calculated and compared using paired samples *t*-test. The mean values of LDH pre-transfusion and post-transfusion of fresh blood in comparison to 7 days banked blood (67.02±101.02 *vs* 107.30±121.69 U/L) showed a bigger increase in post-transfusion LDH when 7-days stored blood was transfused. However, this was statistically not significant (p=0.13). However, there was no significant increase in either direct bilirubin (0.13±0.14 mg/dL *vs* 0.12±0.15 mg/dL, p=0.76) and indirect bilirubin (0.42±0.55 mg/dL *vs* 0.32±0.32 mg/dL, p=0.45). To determine levels of creatinine, glucose, C-reactive protein (CRP), and uric acid were determined to assess the level of body metabolites affected by the storage time of the transfused blood. No difference in creatinine, glucose, and uric acid variations was noted. Interestingly, levels of CRP which is an inflammatory marker showed a significantly higher raise when 7-days stored blood was transfused in comparison with fresh blood. (1.89±2.38 mg/dL *vs* 6.43±7.46 mg/dL, p=0.012) (Table-3).

Table-3: Differences in biochemical parameters after fresh or 7 days stored blood (Mean±SD)

Variables	Difference after transfusion of fresh blood	Difference after transfusion of 7 days stored blood	*p*
LDH	67.02±101.02	107.30±121.69	0.13
Bilirubin (Direct)	0.13±0.14	0.12±0.15	0.76
Bilirubin (Indirect)	0.42±0.55	0.32±0.32	0.45
Creatinine	0.10±0.06	0.07±0.06	0.149
Glucose	28.5±46.8	34.40±108.12	0.76
CRP	1.89±2.38	6.43±7.46	0.012
Uric Acid	0.50±0.511	0.42±0.34	0.52

An increase in serum iron levels was noted in transfusion of both fresh blood (155.79±177.77 ug/dL) and 7-days stored blood (91.76±88.87 ug/dL). However, the differences in the serum iron raise were not statistically significant (paired samples *t*-test, p=0.18). Transfusion of both fresh and 7-days stored blood resulted in increased iron levels in the post-transfusion sample. In fresh blood transfusion, serum ferritin levels increased by 1072.70±1292.052 ng/mL, and in 7-days stored blood transfusion, serum ferritin levels increased by 826.32±1088.94 ng/mL. These differences were statistically not significant (p=0.55) (Table-4).

Table-4: Difference in iron overload between fresh blood and 7 days stored blood

Variables	Difference after transfusion of fresh blood	Difference after transfusion of 7 days stored blood	*p*
Ferritin	1072.70±1292.052	826.32±1088.94	0.55
Iron	155.79±177.77	91.76±88.87	0.18

DISCUSSION

Our study aimed to investigate the differences in haematological and biochemical markers after transfusion of fresh blood compared to 7-days banked blood. The main purpose of blood transfusion in thalassaemia patients is to raise the red blood cells count by suppressing the ineffective erythropoiesis. As per previous studies performed on different sets of patients to determine transfusion-related differences, no significant difference was found between transfusion of fresh blood and up to 15–20 days banked blood.[11] Similarly, we found no significant difference in haematological parameters post-transfusion fresh blood versus 7 days banked blood except non-significant post-transfusion increase in WBCs, platelets, CRP, ferritin, and iron levels. The haemoglobin level was expected to be raised after blood transfusion. According to Linda *et al*, the haemoglobin level raised up to 1g/dL in patients with severe anaemia after transfusion of packed red blood cells.[12] As per our study, an increase in haemoglobin level was observed in both groups

receiving fresh blood and 7 days old blood. No significant increase in MCV, MCH, and MCHC post-transfusion levels were observed in both groups. Although transfusion-dependent thalassaemia patient's red cells have very low MCV, MCH, MCHC, our patients had received multiple transfusions and therefore, their haematological picture was representative of previously transfused blood from healthy donors. Therefore, transfusion of normocytic red cells did not increase MCV, MCH, or MCHC. Similar findings were earlier observed by Spadaro *et al* in anaemic patients post-transfusion blood samples.[13]

In our research study, we also observed a mild but significant post-transfusion increase in WBCs count. The plausible explanation for the increase in WBCs count may be due to the infusion of pro-inflammatory cytokines especially IL-6.[14,15] A mild leucocytosis is observed in patients who are transfused with leuco-depleted blood.[16] Increased in post-transfusion WBCs count was also found by Hirani *et al*.[17] Another proposed mechanism is the increased concentration of non-transferrin bound iron (NTBI) in the blood leading to the production of pro-inflammatory cytokines including IL-6 and IL-8 which causes leucocytosis in the recipient's blood.[18] More recently, Straat *et al*, concluded that RBCs transfusion bags contain extra-cellular vesicles (EVs) as supernatant which may increase due to prolong storage of blood. These EVs cause release of TNF, IL-6, IL-8, and IL-10 which results in the production of a strong pro-inflammatory host response, i.e., leucocytosis.[19]

Consistent with increased white cell count, we also observed a significant increase in serum C-Reactive Protein (CRP) in post-transfusion samples of patients transfused with old blood. This finding is as per results shown by Kapur *et al*.[20] The authors have attributed this increase in CRP to EVs which are found in blood bank stored blood. The EVs have pro-coagulant activity. It may be also due to platelets aggregation because of RBCs membrane damage and thrombocytosis as a consequence of splenectomy. Interestingly, the increased platelets were found more in splenectomized patients than non-splenectomized patients by Trinchero *et al*.[21]

We also tested markers of red cell and tissue destruction (bilirubin and LDH). Although the values of these showed a slight increase in post-transfusion samples from patients transfused with old blood, this was not significant. Blood transfusion-related abnormalities in serum laboratory parameters such as bilirubin and lactate dehydrogenase were demonstrated by Weisen *et al*, in transfusion-dependent patients.[22] A non-significant and temporary increase in bilirubin and lactate dehydrogenase levels was observed in patients receiving two packs of RBCs. The increase in bilirubin level is due to the destruction of non-viable RBCs in the first 1–2 hours after transfusion and conversion of released haemoglobin into bilirubin by the liver. This phenomenon is seen in almost all patients who receive blood. However, the bilirubin level returns to normal after 24 hrs.[23] Hence, reticulocytes count was not performed to detect the significance of increase in bilirubin and LDH levels. Since we tested our post-transfusion samples 24 hours after transfusion, these values might have returned to normal.

Blood transfusion leads to an increase in serum ferritin and iron levels. Increase serum iron concentration causes the transfer of excessive iron to the liver and other organs such as the heart, endocrine glands, and tissues which may lead to severe toxicity and life-threatening medical conditions.[24] Cardiac myopathy is one of the most common pathological conditions that results from iron overload and causes most of transfusion-related deaths.[25] Production of reactive oxygen species (ROS) is another problem related to iron load. These free radicals cause degradation of cellular lipid contents and cell organelles including DNA leading to the death of cells.[26] A patient suffering from thalassemia or other types of haemoglobinopathies receives about 200–250 mg iron per unit of blood. The body cannot excrete excessive iron and therefore the excess iron is managed through iron chelation therapy.[27] Assessment of iron overload is usually performed by measuring serum ferritin level in developing countries. ferritin is an iron storage protein that is used as a marker for analyzing iron load. Serum ferritin, being an acute phase reactant, also increases in inflammatory diseases, liver diseases, renal diseases, and metabolic abnormalities.[28] The increase in iron and serum ferritin levels post-transfusion is observed by different studies. Sadoom *et al*, found a significant increase in serum ferritin and iron levels in the Iraqi population.[29] An interesting finding in our study was a non-significant increase in blood ferritin and free iron level in post-transfusion blood samples of both groups (p=0.55 and 0.18 respectively). Probably, 7-days stored blood caused a more acute increase in serum iron and ferritin in the old-blood transfusion group and the difference was reduced at 24 hours.

CONCLUSION

At least seven days stored blood can be safely transfused to transfusion-dependent thalassemia major. However, due to limitation of our study budget, large-scale study should be designed in future for generalization of our results.

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