Original Article

Comparative Analysis of Storage Lesion of Cord Blood And Adult Homologous Blood; A Base to Establish Cord Blood Banks in Pakistan

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Objective: To evaluate the feasibility of collecting Umbilical Cord Blood (UCB) and its efficacy for autologous transfusion to infants by measuring collected volume and by determining storage related changes in comparison with Homologous Adult Blood (HAB).

Methods: We collected 50 UCB & 50 HAB units on basis of inclusion criteria. Blood volume was calculated for each unit, microbiological cultures were done on 7th day. Samples from each unit were obtained on Days 0, 15 & 30 to be evaluated for hemoglobin, cell counts, hematocrit, hemolysis rate, potassium levels & Red cell ATP levels.

Results: Volume of HAB units was 470±15 ml while that of UCB was 70±25 ml. Bacterial culture were +ve in 2 HAB units (4%) & 5 UCB units (10%). In UCB units; Hb, RBC & K+ levels increased while WBC, PLT counts & ATP levels decreased during standardized storage of 30 days. All these findings were comparable & statistically non-significant to HAB changes except K+ levels which were significantly more in HAB. Checked by paired Student t test, p-value > 0.05. The changes were in permissible limits for transfusion.

Conclusions: The collected volume of UCB was sufficient for at least two transfusions. Provided the collection technique is more sterile, UCB can be collected & stored for at least 30 days in our health-care settings.

Keywords: umbilical cord blood, storage lesions, RBC ATP levels.

Introduction

Although a lot of research work has been done and methods are devised to overcome or at least reduce the adverse effects of blood transfusion but in last 2 decades we witnessed dramatic paradigm shift[1] in which "a product once regarded as one of the great advances in modern medicine is now viewed as potentially harmful".[2,3] Its mainly because of discovery of newer transfusion transmitted (TT) pathogens & problems of non-infectious hazards which are more frequent & more serious.[4,5] In lieu of different hazards of homologous blood transfusion (HBT) scientists turned to the use of autologous blood (AB). Since long, demonstration of effectiveness & safety of autologous blood transfusion (ABT) in adults led to the urge to use it in pediatric patients esp. infants.[6]

Nowadays, due to advances in diagnostics & supportive care, even immature & VLBW neonates survive but rapid senescence of HbF containing RBCs, insufficient erythropoiesis, frequent phlebotomy for investigations & cardio-pulmonary or infectious complications causes sufficient anemia to make these neonates a potential candidate to receive multiple small volume transfusions.[6] Parents are often hesitant for transfusing blood from multiple donors to their newborn, an enigma which is very severe in our country due to strong ethnic & tribal cultural bonding. Many studies have reported that ABT is safe & effective in neonates[7] even in preterm infants.[8,9] Umbilical Cord Blood (UCB) is reported as best source for autologous blood transfusion. Furthermore, it is reported that UCB can be safely used even as homologous source.[10-12]

Placenta (or placental vessels) contains a quarter to a third of newborn blood volume which is currently discarded as waste. The biggest benefit of UCB is probably presence of hematopoietic stem cells (HSCs) which have greater proliferative & colony forming capacity, are more responsive to some growth factors and produce fewer complications if transplanted. Hence a lot of work is going on regarding its usefulness in transplantation in different diseases in place of BMT.[13,14,] But use of allogeneic HSC is limited by the need to find an HLA compatible donor & because constitute only 0.01 % of the nucleated cells of the cord blood, the remaining 99.99% is wasted.[15]

Although, a lot of work has been carried out in some developed countries regarding storage and use of UCB, no documented work has been carried out in Pakistan. Despite ongoing work in this field there is still no consensus on method of collection of cord blood[16] & different researchers have tried different methodology.[17] Furthermore, the studies carried out,

Give contrasting data especially regarding volume of cord blood collected, storage period and rate of bacterial contamination. In this study we intended to assess some storage lesion parameters in order to establish that cord blood can be collected & stored efficiently in our circumstances as well.

Methods

It is a cross sectional analytical study carried out at the, departments of Pathology and Gynecology / Obstetrics and Baqai Hospital Nazimabad laboratory constituents of Baqai Medical University, Karachi. The span of the study was six months (from SEP 2016 to FEB 2017). 50 units of cord blood collected from the umbilical cord of the neonates and 50 units of homologous adult blood (HAB) were also collected to serve as control.

The inclusion criteria for neonates from whom UCB obtained was that they should be live birth, delivered per-vaginum, gestational age between 36 & 40 weeks and have birth weight 2500 gms or more. Neonates of mothers having any obstetrical complication were excluded. HAB was collected as per routine guidelines. The cord blood samples were collected soon after delivery. The expelled placenta was received in a sterile bowl with umbilical end of cord clamped. After stringent cleaning, umbilical cord was wiped with 70% alcohol and an iodine swab at the intended site of insertion of needle into the umbilical vein. Cord blood was obtained in a 250 ml CPDA-1 blood bag, kept 1.0- 1.5 m below the level of placenta; the volume of anticoagulant was adjusted (by removing it into a graduated beaker just prior to inserting the needle into umbilical vein) to maintain a ratio of 1:7, which was 10-14 ml as according to our pilot study the volume of cord blood obtained was 75 -100 ml. After 5-10 minutes when collection completed, the blood bag was double sealed by an electric sealer. Adult homologous blood was obtained from healthy donors as per routine. The volume of the blood obtained was assessed with the help of weighing scale. None of the blood bag was used for transfusion. Blood volume obtained was calculated and blood bags from both the groups were stored under similar conditions at 2-6oC for a maximum of 35 days. Samples were obtained from both groups, about 3 ml from tubing after proper mixing of blood bag, on Day 0, Day 15 and Day 30 for evaluation of Hemoglobin, Hematocrit, Blood Cell Counts, Adenosine Tri Phosphate (ATP) levels, Plasma Potassium (K+) levels and Hemolysis rate.

CBC parameters were performed using automatic hematology analyzer (Sysmex KX-21). As for biochemical parameters; red cell ATP levels were measured spectrophotometrically by plate reader (Digitex, China) using commercial "ATP Colorimetric Assay Kit" (Biovision, USA) as per manufactures procedure. Potassium levels were determined using Microlab chemistry analyzer. Hemolysis rate was calculated through a formula involving measurements of total Hb. and supernatant Hb obtained using spectrophotometer. About 5 ml of blood sample was also taken on 7th day for Microbial Culture. Data was analyzed using SPSS version 17 by employing Paired sample t-test for each quantitative variable and Pearson Chi-Square for qualitative variables comparing both the groups. P value of < 0.05 was considered as significant.

Results

Volume of HAB units was 470±20 ml while that of UCB was 70±25 ml. **(Table-1)** is showing volume of cord blood obtained in stratified form. Bacterial culture came +ve in 2 HAB units (4%) while 5 UCB units (10%) came+ve **(Fig-2).** Regarding the hematological & biochemical parameters of UCB units at different time-intervals; Hb., Hct., RBC & K levels increased while WBC, platelet & ATP levels decreased during storage of 30 days. The findings were comparable with almost similar changes in HAB.

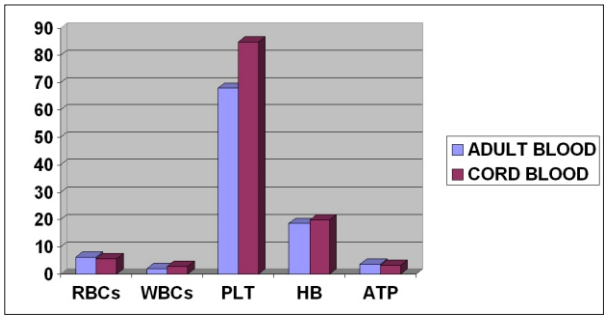

Fig-1: Comparison of HAB and UCB Parameters at 30th day of storage.

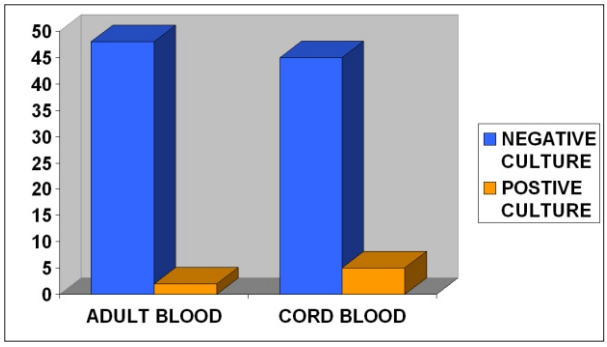

Fig-2: Comparison of bacterial contamination rates of HAB and UCB at15 and day 30.

The readings of the above mentioned parameters of HAB & UCB at 30th day were statistically compared and were found to be non-significant (p-value =>0.05) **(Table-4 and Table-5).** All these changes were in permissible limits for transfusion. Summary of findings is given in **(Table-2 and Table-3)** and depicted graphically in **(Fig-1).**

Table-1: Stratified umbilical cord blood obtained volume.

Stratified cord blood volume	No. of Units (n=50)
31-50	10
51-70	14
71-90	12
91-100	14

Table-4: Comparison of means of storage lesion parameters of HAB & UCB at day 15.

Parameters	HAB	UCB	P-value
Hb (gm/dl)	14.3±1.41	13.9±1.25	0.101
RBC (10^{12}/L)	4.753±0.58	4.63±0.53	0.287
HcT (%)	34.18±3.31	33.36±1.77	0.080
WBC (10^9/L)	3.64±1.03	3.56±1.02	0.702
PLT (10^9/L)	126.4±21.7	134.9±33.2	0.140
ATP (mmol/gm Hb)	4.49±0.66	4.61±0.82	0.379
K (mEq/L)	13.9±1.86	13.2±1.425	0.041
Hemolysis rate (%)	0.33±0.038	0.0325±0.038	0.063

Table-5: Comparison of means of storage lesion parameters of HAB & UCB at day 30.

Parameters	HAB	UCB	P-value
Hb (gm/dl)	15.77±2.4	16.32±1.7	0.186
RBC (10^{12}/L)	5.597±0.56	5.497±0.399	0.318
HcT (%)	34.69±2.95	33.94±2.9	0.161
WBC (10^9/L)	1.42±0.79	1.498±0.76	0.620
PLT (10^9/L)	57.26±12.15	60.42±13.4	0.221
ATP (mmol/gm Hb)	3.88±0.69	4.06±0.78	0.217
K (mEq/L)	23.13±2.1	21.86±1.2	0.003
Hemolysis rate (%)	0.604±0.03	0.56±0.082	0.001

Discussion

The majority of newborns do not need transfusions, however, preterm low birth weight newborns and newborns suffering from anemia (e.g. congenital or acquired) do need transfusions. Many countries have established cord blood banks. Our country being one of those having higher birth rate, have greater potential for establishment of cord blood banking. Therefore in this study we tried to determine the feasibility of collecting CB in order to pave road towards establishment of cord blood banks.

Unlike some studies in which they got very less amount of CB (e.g. 49.7±18.7 ml by Garitsen et al),[18] in our study, we got about 70 ± 25 ml which is sufficient for at least two transfusions in accordance to Transfusion Guidelines, UK 7TH ed. 2005. About 10-20 ml /Kg body weight is standard for one episode & neonates usually require 2-3 episodes of transfusions.[6] Among hematological parameters (UCB units) Hb, Hct, RBC, hemolysis rate showed increase while WBC & PLT showed decrease during the storage for 30 days. All these changes are within the permissible range for transfusion.[19] These changes when compared with controls, i.e. Homologous Adult Blood (HAB) stored simultaneously under same conditions as for UCB, came out to be non-significant, p value for comparison of Hb=0.101 at day 15th & 0.186 at day 30th, for RBC = 0.287 & 0.318, for Hct=0.080 & 0.161, for WBC = 0.702 & 0.620, for PLT=0.140 & 0.221 respectively. The rise in RBC counts after storage was probably due to mild hemolysis and fragments were counted as individual RBCs by the automated cell counter while rise of Hb value was due to rise in free plasma level again due to mild hemolysis. A finding which needs further assessment through large-scale studies especially in our country. As for hemolysis rate; comparison at day 15 came out to be non-significant (p value 0.063) but on day 30 comparison it came out as significant (p value 0.001) the reason for which was more hemolysis in HAB units suggesting that UCB cells are more stable. All these findings are comparable to other studies.[18,20]

References

Table-2: Summary of storage lesion parameters of HAB* stored in CPDA-1 (as Mean ± S.D).

Storage Days	RBC (10^{12}/L)	WBC (10^9/L)	PLT (10^9/L)	Hct (%)	Hb (gm/dl)	ATP (mmol/gmHb)	Plams K+level (mEq/L)	Hemolysis rate (%)
0	5.4±0.8	6.7±1.4	265±70	48.5±1.0	13.5±1.1	4.8±0.6	5.82±1.05	0.086±0.025
15	5.8±0.6	4.5±0.9	170±50	50.4±0.8	15.7±0.7	4.1±0.4	13.9±1.86	0.33±0.038
30	6.3±1.0	2.1±1.0	68±42	53.2±0.9	18.6±1.0	3.7±0.8	23.13±2.1	0604±0.0296

**HAB = Homologous Adult blood*

Table-3: Summary of storage lesion parameters of UCB* stored in CPDA-1 (as Mean ± S.D).

Storage Days	RBC (10^{12}/L)	WBC (10^9/L)	PLT (10^9/L)	Hct (%)	Hb (gm/dl)	ATP (mmol/gmHb)	Plams K+level (mEq/L)	Hemolysis rate (%)
0	4.9±1.2	8.1±2.3	28±62	46.2±1.3	14.4±1.5	4.9±0.5	5.9±1.2	0.0934±0.025
15	5.2±0.9	5.0±1.9	185±60	48.8±0.9	16.2±1.0	4.0±1.0	13.2±1.43	0.325±0.04
30	5.7±1.1	2.8±1.5	85±68	514±1.1	19.9±1.2	3.2±1.2	21.86±1.2	0.592±0.034

**HAB = Homologous Adult blood*

Studies regarding usefulness of cord blood either used few hematological parameters or has not combined them with biochemical parameters. While In our study; we analyzed more hematological parameters and have assessed biochemical parameters in combination. Also our study suggested that UCB can be safely stored for 30 days in CPDA-1 which is a better result as compared to some other studies which recommended 14 days & 28 days storage[18]. The bacterial contamination rate was 10 % in our study which is mainly due to use of open system & less trained staff but it is again not a bad result as some studies have reported more than it (e.g. 15.8 % by Surbeck et al) but it does need rectification as studies have reported lesser rates[21]. The values of Day 0 of UCB in our study can be used as baseline. Although our sample size was small; these values can't be proposed to represent the population, but they can serve as a reflection of normal pattern as to our best knowledge there is no other published data found addressing normal hematological & biochemical parameters of UCB of Pakistani population.

Conclusion

The collected volume was sufficient for at least two transfusions. So, making the collection technique more sterile, UCB can be collected & stored for at least 30 days and thereby can be used as an alternative source of blood instead of adult homologous blood for transfusions in neonates.

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