Evaluation of the Affect of Maternal and Neonatal Factors on Cord Blood Parameters?

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Background: The number of nucleated cells (NC) infused into the recipient is highly correlated with the probability and speed of cord blood (CB) hematopoietic stem cell transplantation; therefore it is necessary to obtain CB units with sufficient NCs.
Study design and methods: Cord blood was collected from normal vaginal and cesarean deliveries with placenta in uterus and immediately processed and assessed for volume NC count, $CD34^+$ cell count and CFU-GM. These parameters were then processed and analyzed to find out whether they are correlated with maternal and neonatal characteristics such as mother's age, parity, gestational age, babies birth weight, and sex.
Results: CB had a higher volume in samples taken from cesarean deliveries with an open collection method in comparison with samples collected from vaginal deliveries with a closed collection method. No significant correlation was found between maternal-neonatal factors and CB parameters in vaginal deliveries; while in cesarean cases, male newborns have a higher volume and cell count. In multiparous women, CB cell counts are higher than women who have a parity of 1 or 2 (ALL $p < 0.05$). Volume reduction with a ratio of one part hespan to five parts blood recovered more NC (88%) than a ratio of one to seven (73%).
Conclusion: : It can be concluded that CB parameters were influenced by the collection method, processing technique, maternal and neonatal factors.

Key words: cord blood, nucleated cell, neonatal factors, maternal factors

Introduction:
Hematopoietic stem cell transplantation (HSCT) is recommended, (and sometimes even the sole treatment) for hematological malignancies, bone marrow failure syndromes, hereditary immunodeficiency states and metabolic disorders.[1] Bone marrow and peripheral blood have so far been two suitable sources for transplantation; however, because of the absence of fully HLA matched donors and the high risk of GVHD, only a few patients can benefit from these sources.[2] Hence, for the first time in 1988, cord blood (CB) was used as an alternative source of HSCT in a child with Fanconi anemia,[3] which had several benefits such as ability to tolerate HLA mismatched transplants,[4,5] a lower risk of acute and chronic GVHD[6,8] and lower risk of blood-transmitted infectious diseases. However, there are some restrictions in CB applications as well, i.e. CB volume is small and collection can not be repeated. Therefore, the number of cells collected from each sample is limited. On the other hand, the number of nucleated cells (NCs) infused per kg of recipient body weight is strongly correlated with neutrophil recovery time.[9,10] Since the lowest safe dose of NCs to provide durable engraftment is 2×10^7NC/Kg;[11] the number of infused cells is far below the recommended dose of bone marrow cells and peripheral blood stem cells.[12] In other words, only a small number of CB collections contains sufficient cells for adults.[13] In this study, we want to evaluate the effects of maternal and neonatal factors on CB parameters, to select the best samples before collection; and we want to define the distribution of CB units according to assumed recipient body weight for CB banking.

Materials and methods:
1- Collection of CB units: Cord blood was collected from 56 normal vaginal and 42 cesarean deliveries before the placenta delivery.[14,15] In vaginal deliveries (VDs) after birth, the cord was clamped in two places, 7 and 5 cm, from the newborn. Then the cord was cut after disinfection, a needle was inserted into the umbilical vein above the clamp and blood was drained via gravity into the sterile collection bag, containing 25 ml Citrate Phosphate Dextrose Adenine (CPDA) as an anticoagulant agent. In cesarean deliveries (CDs), after the clamping and cutting the cord, it was inserted into a sterile glass container with 25 ml anticoagulant. Blood flowed into the glass by gentle milking.
2-Cord blood processing: Within 1-2 hours after collection and taking samples for laboratory testing, the CB volume was measured and the white cells were separated from the red cells using 6% Hydroxy Ethyl Starch (HES) in 0.9% sodium chloride [15,16] (from Fresenius). HES was added to the CB collection bag with the ratio of one part HES to five parts blood[17] in vaginal CB units, and 1 to 7 in cesarean CB units. The bag was incubated for one hour at room temperature to separate red cells from white cells. After an hour, white cell-rich plasma was slowly transferred into an attached transfer bag by a plasma expresser.
3-Laboratory Assays: The NC count was performed by hematological cell counter (MS9 Melet). The Viability was tested with Trypan Blue; dye exclusion method.

The $CD34^+$ cells were measured with flow cytometric analysis (Epics XL-MLC Coulter). Colony forming cell (CFC) was assayed by semisolid culture[18,19] containing: 10^5 cell/ml, 0.3% Agar, 20% FCS (Gibco), 100U/ml Penicillin, 100µg/ml Streptomycin (Gibco), 50 ng/ml GM-CSF(Serva), 20 ng/ml SCF(R&D) in IMDM (Iscove's Modified dulbecco's Medium. from Gibco).

Data collections and analysis:

Mothers' characteristics such as age, weight, the number of previous live births (parity), and gestational age were recorded in enquiry forms at the bedside before the delivery. Infants' gender and weight were measured after delivery.

The effect of the maternal and neonatal factors on CB parameter was examined by univariate analysis. We used the independent sample test for quantitative data and chi-square test for qualitative data to compare CB parameters in the presence of each particular maternal and neonatal character rustics.

Results:

Mothers' and neonates' characteristics are shown in table 1.

Table1. Mothers and neonates characteristics in vaginal and cesarean deliveries.

Characters	Delivery	No.	%	Mean+ std	Range
Maternal	VDs			24.16+4.78	17-38
Age (year)	CSs			27.55+3.98	20-35
Maternal	VDs			73.25+9.58	56-94
Weight(Kg)	CSs			77.44+13.6	57-110
Gestational	VDs			38.48+1.99	33.1-41
Age (week)	CSs			38.82+0.83	37.4-40
Parity 1	VDs	40	71.4		
2		9	16.1		
3		7	12.5		
1	CSs	7	17		
2		10	24		
3		17	41		
4		8	17.9		
Sex of baby	VDs				
F		20	35.7		
M		36	64.3		
	CSs				
F		20	47.6		
M		22	52.4		
Weight of baby (g)	VDs			3047.35+390.11	2050-3800
	CDs			3050.35+444.13	2250-4250

In VDs there is no significant correlation between CB parameters and maternal or neonatal factors. However, in CDs, CB volume was positively correlated with the birth weight of the newborns (p= 0.01). The male neonates' mean weight was significantly higher than females (3388 vs. 2917 g respectively, p= 0.004). The CB volume and the NC count were significantly higher in male neonates than females (p= 0.02, p= 0.07). The outcome measures were not influenced by maternal or gestational age. Parity more than 2 (3 or 4) had a significant positive effect on the nucleated cell count (p= 0.007). The viability of all samples was over 98%. The outcomes of CB parameters is shown in Table 2.

After processing CB nucleated cell recovery rate was 88% in VD units and 73% in CD units.

The distribution of collected CB units for assumed recipient weight is shown in table 4.

Table 2.The outcome of CB parameter in vaginal and cesarean deliveries

Caracters	Del.	Mean +std.	median	Range
CB Volume (ml)	VDs	72.96+18.78	73.50	40-129
	CDs	86.34+18.37	90.00	50-140
Total nucleated cell count Before processing ($\times10^7$)	VDs	82.12+43.50	72.57	24.51-239.40
	CDs	72.83+28.27	70.00	18-168
Total $CD34^+$ cell Coun $x10^5$	VDs	20.98+16.06	17.00	0.57-85.90
	CDs	Not done		
Total CFU count $\times10^4$	VDs	23+18.73	57.43	1.17-389.62
	CDs	Not done		
Total nucleated cell Count after processing $\times10^7$	VDs	72.88+33.64	63.73	25.50-154.80
	CDs	53.10+22.80	47.00	20-110

Table 3: Distribution of CB units that contain minimum $2x10^7$ NC/kg recipient body weight.

Recipient Weight	Vaginal CB $2x10^7$NC/kg	Caesarian CB $2x10^7$NC/kg
<20kg	100%	100%
21-30kg	89%	90%
31-40kg	62.5%	61%
41-60kg	37.5%	29%
61-100kg	19.6%	7.3%
>100kg	1.8%	0%

Discussion:

Umbilical C.B has been recently considered a useful alternative source of hematopoietic progenitor cells for clinical application.[20,22] The main difference between CB and bone marrow is the smaller number of cells obtained in the CB product. As a result, until now, CB has been used primarily for children. Some ways to resolve this problem consist of: screening and selection of proper CB donors before collection, choosing the best methods for collection, increasing the recovery rate of CB processing and ex vivo expansion of CB.

In our study, CB volume was significantly higher in CDs than in VDs (p<0.001). However, although NC count was higher in vaginal delivery than cesarean, it was not significant.

Sparrow et al,[23] showed a significantly higher CB volume in cesarean than in vaginal deliveries (Median volume 76 vs. 63 ml, p<0.0001), but they did not report significant differences between VDs and CDs in the total number of nucleated cells.

No significant correlation was found between maternal and neonatal factors and CB parameters in VDs. However, in CDs, heavier newborns had higher CB volumes and cell counts (p<0.02, 0.07 respectively). In comparing VDs and CDs neonatal factors, it was found that the newborns' mean weight in CDs were higher than VDs. May be this significant correlation exists only in heavier neonates.

Ballen et al,[24] asserted that heavier newborns had higher NC counts, CD34+ and CFC, as well. They also stated that newborns of longer gestational age had higher nucleated cell counts but lower CD34+ cell and CFC count; however, this was not reconfirmed by the present study.

We noticed that multiparous women in CDs had higher NC counts ($p<0.007$), opposingly, Ballen et al,[24] had shown that women with fewer previous live births had higher cell counts and more CD34+ and CFC.

Since in VDs, NCs' recovery rate with HES method (in a ratio of 1 unit HES to 5 units blood) was 88% and in CDs (with the ratio of 1 to 7), the recovery rate was 73%, we recommend that the ratio of 1 unit of HES to 5 units of CB was more efficient in the recovery process than the 1 to 7 ratio. Kolger et al,[25] showed 85% recovery rate for NCs with the ratio of 1 HES to 5 blood.

We noticed that the most important factors which affect CB volume were the clamping time and clamped places on the cord. We can collect the highest volume of CB if the cord is clamped during maximum of 15–30 seconds after birth and the first clamp is located at a maximum of 7cm from the infant, with a 5 cm distance between two clamps.

Furthermore, it was noticed that, in the closed collection method compared to the open method, although lower volumes were collected, it had a lower risk of contamination. However, in the closed method with sterile needles, because of the clotting probability on the way to the needle, (in order to collect more volume), cord puncturing was needed, which could increase the rate of infection.

In the distribution of CB units, our results showed that 100% of the units have the lowest safe dose of NCs for recipients less than 20 kg, 60% for less than 40 kg, but less than 20% are suitable for 60-100 kg.

Finally, it can be concluded that almost half of the CB units were suitable for over 40 kg recipients, however, it is necessary to pay more attention to improving the processing and freezing methods in order to recover more NCs.

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