Influence of Mode of Delivery on the Quality of Umbilical Cord Blood Stem Cells

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Abstract: Stem cells have the remarkable potential to develop into many different cell types in the body. Serving as a sort ofrepairsystem for the body, they can theoretically divide without limit to replenish other cells as long as the person or animal is still alive. Umbilical cord blood (UCB) can be used as a source of primitive hematopoietic stem and pluripotent progenitor cells in clinical application to reconstitute the hematopoietic system and/or to restore immunological function in vivo. Compared to hematopoietic stem cells from marrow, UCB has many known advantages. Obstetric factors including gestational age, parity of the mother, sex and birthweight of the newborn, weight of the placenta, duration of labor, and the mode of delivery are known to influence the cell content of UCB. The aim of the present study is to study the influence of mode of delivery on the quality of cord blood stem cells assessed by measuring Total nucleated cell (TNC) count, CD34 Cell count and cell viability. The current study was comparative study thatcarried out on 30 Cord Blood (CB) samples divided into two groups:- **Group A:** 15 cord blood samples will be taken from vaginal deliveries. **Group B:** 15 cord blood samples will be taken from cesarean section (regardless to the cause of cesarean section). After obtaining informedconsent from participating mother attending Alzahra University hospital Department of Obstetric and gynecology for delivery. In conclusion cesarean delivery of neonates would lead to an increase of UCD volume and CD34 counts in UCB compared to those that were collected by vaginal delivery of newborns.

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1. Introduction

The study and use of stem cells have made strides across many fields in medicine. This has included work in hematology, ophthalmology, spinal cord injury, burn therapy, cardiac ischemia, and more recently, in pelvic floor dysfunction ***(Felicia et al., 2012).***

Placental cord blood (CB) contained multipotent hematopoietic stem progenitor cells, similar to those found in bone marrow. It is widely used as an alternative source for transplantations because it can be harvested with no risk to mothers or neonates ***(Omori et al., 2012).***

Umbilical cord blood (UCB) transplants have several strengths including immediate availability, low risk of graft-versus-host disease, level of hematopoietic progenitor cells and ease of Human Leukocyte Antigen (HLA) matching requirement ***(Laughlin et al., 2004).***

Since the first CB transplantation was successfully performed in Paris in 1988***(Glukmanet al., 1989),*** the use of CB has been increasingly encouraged for the patients with hematopoietic malignancies and some hereditary diseases ***(Wuet al., 2006).***

In 2006, more than 10 percent of allogeneic stem cell transplantation in the United States were generated from UCB. Its use and need in the treatment of malignant and non malignant hematologic and immunologic disease are increasing steadily ***(Gwendolin et al., 2008).***

Heamatopietic stem/progenitor cells in CB are also used for various research studies, such as investigation for drug sensitivityor radiation sensitivity, furthermore mesenchymal stem cells in CB have been extensively studied for biomedical therapies and regenerative medicine ***(Omori et al., 2012).***

It is necessary to precisely clarify the individual differences in CB quality more in order to predict its proliferation and engraftment capacities after transplantation.

This isbecause of one of the most serious issues affecting the present standard policy of CB banking is the running costs.

This includes the high cost of personnel, cell processing, and freezing/storage of CB units ***(Nakagawa et al., 2004).***

The most important factors for the outcome of UCB transplantation are total nucleated cell (TNC)

number, CD34 cells, and colony forming units (CFUs) ***(Wagner et al., 2002).***

Studies have also shown that the quality of the collected CB units may be influenced by many obstetric factors.

These factors includethe mothers ethnicity, neonatal and placental weight, gestational age, sex of neonate, length of umbilicalcord, and experience of collection operators ***(Askariet al., 2005).***

The mode of delivery and duration of labor are known to influence the content of UCB ***(Mncinelli et al., 2006).*** It has been shown that the number of UCB stem cell is increased in cesarean section compared to vaginal deliveries ***(Kurtzberget al., 2005).*** However compared to vaginal delivery some studies found high UCB volumes but insufficient TNC numbers after primary elective cesarean sections. Furthermore, stress during vaginal delivery is known to have a positive impact on the content of UCB ***(Lim et al., 2000).***

Aim of the work

To study the impact of mode of delivery on the quality of cord blood stem cells. This could be achieved by measuring Total nucleated cell (TNC) count, CD34 Cell count and cell viability.

2. Patients and method

This comparative study was carried out in collaboration between Obstetrics and Gynecology Department, Faculty of Medicine for Girls Al Azhar University and Reproductive Health Department at National Research Centre. The study was carried out during period from 2013 till 2015.

This studywas carried out on 30 Cord Blood (CB) samples divided into two groups:

Group A: 15 cord blood samples were taken from spontaneous vaginal deliveries.

Group B: 15 cord blood samples were taken from cesarean section (regardless to the cause of cesarean section).

Informed consent was obtained from all mothers in accordance with the Research Ethical Committee (REC) of Obstetrics & Gynecology Department, Al Zhraa University Hospital and Ethical Committee of National Research Centre guidelines and Islamic conference guidelines on stem cell research.

Full history including the number of previous live birth, detailed information on family medical background (for execlusion of Diabetis, Hypertension, or any immunological diseases), genetic history, as well as general information (i.e. drug exposure, travel and sexual histories, etc.) of the donating mothers.

This full history taking was followed by general and local examination and ultrasound was performed and infants were delivered according to normal obstetric practices.

Inclusion criteria:

Singleton, healthy, full-termpregnancies

Execlusion criteria:

Preterm, multiple, unhealthy, high risk pregnancies, presence of congenital fetal malformation and patients withfamily history of congenital diseases.

Obstetric and neonatal factors:-

For each newborn delivery, the following obstetric factors were considered:

- Maternal age (year).
- Gestational period (week).
- Mode of neonatal delivery (vaginal or caesarean delivery).
- Parity (1 to4).
- Neonatal factors included:-
- Infant's genders (male or female).
- Birth weight (gm).
- Length of umbilical cord (cm).
- Cord blood volume (gm)
- Placental weight (gm) were taken into account.

Stem cell Collection:

After complete delivery of the baby and clamping of umbilical cord before expulsion of placenta, the umbilical cord was sterilized with povidone iodine and a 16-gauge needle from a blood-donor set containing 25 ml Citrate Phosphate Dextrose solution as anticoagulant was inserted into the umbilical vein. Cord blood was allowed to flow by gravity, and the needle was removed when blood flow ceased.

From each sample aliquot (about 3ml) UCB was sent to medical laboratory (colour) for CD34+ cell and TNC enumeration within 24-48 hours of collection.

Stem Cell Preparation:

Cord blood samples are evaluated by flow cytometry using a combination of fluorescein isothiocyanate (FITC)-conjugated anti-CD45, phycoerythrin (PE)-conjugated anti-CD34 monoclonal antibodies and 7- aminoactinomycin D (7-AAD) dye for viability assessments as provided by the manufacturer.

High-Yield Lyse is a premixed, fixative-free erythrocyte lysing solution that can be used to eliminate red cells from whole blood for flow cytometric analysis with minimal loss of rare blood cell populations. Using this reagent, lysis of erythrocytes is performed immediately following staining of the blood samples with Invitrogen's fluorochrome-conjugated monoclonal antibodies. There is no need for a wash step.

Antibody Staining and Lysis Procedure:

1. The addition of the lysing solution. This avoids the loss of rare populations of cells. For each

sample to be analyzed, the appropriate volume of conjugated antibody was added to a 5 ml, mm tube.

2. 5micron of fluorescein isothiocyanate (FITC)-conjugated anti-CD45, phycoerythrin (PE)-conjugated anti-CD34 monoclonal antibodieswas added to 1ml of 7- aminoactinomycin D (7-AAD) dye for viability.

3. Appropriate volume of whole blood was pipetted into each tube containing the conjugated antibody or isotype control.

4. Each tube was gently shaked by vortex, and incubate for 15 minutes in the dark at room temperature.

5. 2 ml of High-Yield Lysewas addedto each tube.

6. The tubes were mixed and covered with Parafilm. And incubated in the dark for 10 minutes at room temperature. It is recommended that the tubes be mixed periodically during incubation.

7. Flow cytometer analysis was done according to the manufacturer's instructions. The samples should be analyzed within 3 hours of preparation. Samples should be refrigerated at 4°C if analysis is delayed.

This procedure allows the processing of samples without the need to wash the cells after the addition of the lysing solution. This avoids the loss of rare populations of cells.

Statistical Analysis

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013.

Descriptive statistics were done for quantitative data as minimum & maximum of the range as well as mean±SD (standard deviation) for quantitative parametric data, while it was done for qualitative data as number and percentage.

Inferential analyses were done for quantitative variables using 95% confidence interval, independent t-test in cases of two independent groups with parametric data, Chi square test for differences between independent proportions. While correlations were done using Pearson correlation for numerical parametric data and partial correlation in controlling certain conditions.

The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant. The p-value is a statistical measure for the probability that the resultsobserved in a study could have occurred by chance.

3. Results

I. Demographic data of both groups

Table (1): Comparison between vaginal and cesarean groups regarding maternal characteristics.

Variables		Vaginal (N=15)	Cesarean (N=15)	P
Age (years)	Mean±SD	25.9±4.3	27.1±3.5	^ 0.408
	Range	18.0–35.0	20.0–34.0	
Parity (n, %)	Primigravida	6 (40.0%)	4 (26.7%)	# 0.439
	Multigravida	9 (60.0%)	11 (73.3%)	

No significant difference between vaginal and cesarean groups regarding**maternal characteristics.**
^Independent t-test, #Chi square test

Table (2): Comparison between vaginal and cesarean groups regarding infant characteristics.

Variables		Vaginal (N=15)	Cesarean (N=15)	P
Sex (n, %)	Male	11 (73.3%)	8 (53.3%)	# 0.256
	Female	4 (26.7%)	7 (46.7%)	
GA (Weeks)	Mean±SD	39.6±1.4	38.8±1.4	^ 0.126
	Range	37.0–42.0	36.0–41.0	
Fetal weight (kg)	Mean±SD	3.2±0.6	3.1±0.7	^ 0.650
	Range	2.5–4.5	2.0–4.0	
Placental weight (gm)	Mean±SD	673.3±234.4	666.7±244.0	^ 0.940
	Range	400.0–1100.0	400.0–1100.0	

No significant difference between vaginal and cesarean groups regarding infant characteristics.
^Independent t-test, #Chi square test.

Table (3): Comparison between vaginal and cesarean groups regarding cord characteristics.

Measures	Vaginal (N=15)	Cesarean (N=15)	Difference (Vag.– Ces)	P
Cord length (cm)				
Mean±SD	61.3±12.3	65.7±12.8	-4.4±4.6	^ 0.345
Range	40.0–75.0	50.0–85.0	--	
95% CI	54.5–68.1	58.6–72.8	-13.8–5.0	
Cord blood volume (ml)				
Mean±SD	65.9±14.1	78.5±16.9	-12.7±5.7	^ **0.034** *
Range	45.0–90.0	55.0–105.0	--	
95% CI	58.0–73.7	69.2–87.9	-24.3–-1.0	

CI: Confidence interval, ^Independent t-test

Table (4): Comparison between vaginal and cesarean groups regarding TNC, CD34+ and CD45+ counts.

Measures	Vaginal (N=15)	Cesarean (N=15)	Difference (Vag.– Ces)	P
TNC count (x10^3)				
Mean±SD	23.3±8.2	21.6±8.0	1.7±3.0	^ 0.574
Range	9.7–30.0	6.5–30.0	--	
95% CI	18.7–27.8	17.1–26.0	-4.4–7.7	
CD34+ (x10^3)				
Mean±SD	1.7±1.6	2.3±2.2	-0.5±0.7	^ 0.435
Range	0.0–4.8	0.0–6.5	--	
95% CI	0.8–2.6	1.1–3.5	-2.0–0.9	
CD45+ (x10^3)				
Mean±SD	2.4±1.4	1.5±1.5	0.9±1.1	^ 0.417
Range	0.0–9.3	0.0–9.3	--	
95% CI	0.5–4.3	0.1–2.9	-1.3–3.1	

CI: Confidence interval, ^Independent t-test

Table (5) shows that: No significant difference between vaginal and cesarean groups regarding**cord length. Cord blood volume** was significantly higher among cesarean group than among vaginal group**.**

Table (5): Comparison between vaginal and cesarean groups regarding CD45+ & CD34+ and CD34+ & CD45- counts

Measures	Vaginal (N=15)	Cesarean (N=15)	Difference (Vag.– Ces)	P
CD45+ & CD34+ (x10^3)				
Mean±SD	1.7±2.6	1.1±1.9	0.7±0.8	^ 0.432
Range	0.0–8.0	0.0–7.4	--	
95% CI	0.3–3.2	0.0–2.1	-1.0–2.4	
CD34+ & CD45-				
Mean±SD	2.1±1.7	2.6±2.6	-0.4±0.8	^ 0.592
Range	0.0–5.8	0.0–7.6	--	
95% CI	1.2–3.1	1.1–4.0	-2.1–1.2	

CI: Confidence interval, ^Independent t-test

Table (6) shows that: No significant difference between vaginal and cesarean groups regarding **TNC count. CD34+** was non-significantly higher among cesarean group than among vaginal group**. CD45+** was non-significantly lower among cesarean group than among vaginal group**.**

Table (7) show that: **CD45+ & CD34+** count was insignificantly lower among cesarean group than among vaginal group **and** CD34+ & CD45- count wasinsignificantly higher among the same group.

There were significant positive correlations between cord blood volume and **Cord length, Infant weight, Placenta weight as well as CD34 +** in vaginal and cesarean groups**.** There were significant positive correlations between cord blood volume and **CD45+ & CD34+ as well as CD45- & CD34 +** in cesarean group**.** No significant correlation between cord length and other variables in vaginal and cesarean groups.

Table (8) shows that: No significant difference between vaginal and cesarean groups regarding viability%. Viabilityis often defined as the number of healthy cells in sample **(Martin, 2011).**

Table (6): Comparison between vaginal and cesarean groups regarding viability %.

Measures	Vaginal (N=15)	Cesarean (N=15)	P
Mean±SD	31.0±45.4	28.9±43.3	^ 0.896
Range	0.0–97.0	0.0–97.0	
95% CI	5.9–56.1	4.9–52.8	
Difference (Vaginal – Cesarean)			
Mean±SD	**95% CI**		
2.1±16.2	-31.0–35.3		

CI: Confidence interval, ^Independent t-test

Table (7): Correlation between cord blood volume & length and other variables in vaginal and cesarean groups.

Variables	Measures	Blood volume ^		Cord length #	
		Vaginal	CS	Vaginal	CS
Cord length	R	0.966	0.964		
	P	**<0.001***	**<0.001***		
Maternal age	R	-0.222	0.002	-0.315	0.074
	P	0.426	0.993	0.273	0.802
Infant GA	R	-0.119	0.123	0.875	-0.859
	P	0.673	0.661	0.125	0.141
Infant weight	R	0.824	0.980	0.114	0.692
	P	**<0.001***	**<0.001***	0.886	0.308
Placental weight	R	0.822	0.976	0.030	0.836
	P	**<0.001***	**<0.001***	0.970	0.164
TNC count	R	0.399	0.440	-0.395	0.458
	P	0.141	0.101	0.605	0.542
R1	R	0.388	0.694	0.174	-0.145
	P	0.153	**0.004***	0.826	0.855
CD34+	R	0.933	0.976	0.927	0.422
	P	**<0.001***	**<0.001***	0.073	0.578
CD45+ & CD34+	R	-0.075	-0.513	0.049	0.932
	P	0.792	**0.050***	0.951	0.068
CD45- & CD34+	R	0.240	0.963	0.868	0.346
	P	0.390	**<0.001***	0.132	0.654
CD45+	R	0.039	-0.452	-0.226	-0.814
	P	0.889	0.091	0.774	0.186
Viability	R	-0.053	-0.213	0.233	-0.267
	P	0.850	0.445	0.423	0.356

^Pearson correlation, #Partial correlation (controlled for cord blood volume), *Significant

Table (8): Correlation between maternal age & infant GA and other variables in vaginal and cesarean groups

Variables	Measures	Maternal age^		Infant GA^	
		Vaginal	CS	Vaginal	CS
Infant weight	R	-0.115	-0.061	-0.251	0.089
	P	0.684	0.829	0.367	0.752
Placental weight	R	-0.044	0.048	-0.059	-0.062
	P	0.875	0.866	0.836	0.827
TNC count	R	-0.214	0.209	-0.062	-0.015
	P	0.445	0.454	0.825	0.958
R1	R	-0.563	0.360	0.068	-0.099
	P	**0.029***	0.187	0.809	0.726
CD34+	R	-0.185	0.039	-0.092	0.021
	P	0.510	0.892	0.745	0.940
CD45+ & CD34+	R	-0.160	0.076	-0.022	-0.449
	P	0.568	0.789	0.937	0.093
CD45- & CD34+	R	0.092	0.054	0.210	0.033
	P	0.745	0.848	0.453	0.908
CD45+	R	-0.605	0.374	0.049	0.145
	P	**0.017***	0.169	0.861	0.605
Viability	R	0.208	0.157	-0.107	0.102
	P	0.475	0.577	0.704	0.719

^Pearson correlation, *Significant

There were significant negative correlations between maternal age and **CD 45** in vaginal group only.

Table (9): Correlation between infant weight & placental weight and other variables in vaginal and cesarean groups.

Variables	Measures	Placental weight ^		Infant weight #	
		Vaginal	CS	Vaginal	CS
Infant weight	**R**	0.930	0.954		
	P	**<0.001***	**<0.001***		
TNC count	**R**	0.460	0.464	0.822	-0.362
	P	0.084	0.081	0.178	0.638
R1	**R**	0.292	0.730	0.797	-0.245
	P	0.292	**0.002***	0.203	0.755
CD34+	**R**	0.924	0.979	-0.195	0.887
	P	**<0.001***	**<0.001***	0.805	0.113
CD45+ & CD34+	**R**	-0.179	-0.401	0.356	-0.373
	P	0.524	0.139	0.144	0.127
CD45- & CD34+	**R**	0.481	0.963	0.180	0.959
	P	0.070	**<0.001***	0.820	**0.041***
CD45+	**R**	-0.128	-0.489	0.484	0.258
	P	0.650	**0.050***	0.516	0.742
Viability	**R**	0.228	-0.190	0.806	-0.376
	P	0.413	0.498	0.194	0.624

^Pearson correlation, #Partial correlation (controlled for placental weight), *Significant

There were significant positive correlations between placental weight and **infant weight as well as CD34+ in both groups.** There were significant positive correlations between placental weight and **CD45- & CD34+ in cesarean group.** There was a significant negative correlation between placental weight and **CD45+.** There was a significant positive correlation between **infant weight and CD45- & CD34+ in cesarean group.**

4. Discussion

Once considered a biological waste product and generally discarded after delivery, CB has emerged as a viable source of hematopoietic stem cells for transplantation ***(Atsuko et al., 2012).***

This source of stem cells has been successfully used to replace bone marrow transplantation. Cord blood has several advantages over adult hematopoitic stem cell sources. These include

1-Ease and safety of procurement,

1- Rapid availability,

2- No donor attrition,

3- Decreased viral transmission,

4- Unlimited supply and increased ethnic representation,

5- Abundance of hematopoitic progenitor cells.

6- Enhanced *in vitro* proliferative and self renewal capacity.

7- Immaturity of T-cell mediated immunity, reduced graft versus host disease (gvhd) and diminished need of HLA matching ***(Tulika et al., 2011).***

The main difference between cord blood and bone marrow is the smaller number of cells obtained in the cord blood product. As a result, until now, cord blood has been used primarily for children. Some ways to resolve this problem consist of screening and selection of proper cord blood donors before collection, choosing the best methods for collection, increasing the recovery rate of cord blood processing and ex vivo expansion of cord blood ***(Tulika et al., 2011).***

Furthermore, a number of factors have been described that may influence the total volume collected, quantification of UCB CD34+cells, andthat may account for the variations in the reported results. Some studies showthat cesarean deliveries provides collection of a higher volume of cord blood than vaginal deliveries. They also stated that higher cord blood volume is correlated with high concentration of CD34+ cells ***(Yamada et al., 2000).***

However previous studies reported that the mode of delivery has no impact on CB yield ***(Lim et al., 1994).***

This study was carried out on 30 umbilical cord blood samples, 15 of them were delivered by spontaneous vaginal delivery & 15 were delivered by cesarean section.

In present study as regarding the maternal chracteristics The mean maternal age was 25.9+4.3 years (range 18.0-35.0) in vaginal group and 27.1+3.5 (range 20.0-34.0) in cesarean group with no statistical significant difference between both groups (P= 0.408).

These findings in contrary with ***Yassin & Saida., 2012*** whoincluded 7916 women and about 14.7%

delivered by cesarean section and foundthat maternal age was significantly associated with cesarean delivery and The probability of cesarean deliveries among women aged 30 years and older was 0.8 that for women younger than 30 years.

The decisive factor to be considered in the relation between maternal age and cesarean delivery in Egypt is the proportion related to obstetricians' attitude, behavior and practice patterns. Obstetricians might opt to unnecessary cesarean deliveries because they can be convenient and lucrative. This is particularly true for the private sector in which guidelines for indications of cesarean section not followed.

As regarding to parity; primigravidas were 6(40.0%) and 4(26.7%) in vaginal and cesarean group respectively. Whereas multigravidas were9(60.0%) and 11(73.3%) in vaginal and cesarean group respectively with no statistical significant deference between both groups (p= 0.439).

These findings were in contrary with ***Yassin & Saida., (2012)*** who found that parity was significantly associated with cesarean delivery, women with less than three live births were two times more likely to undergo cesarean delivery than women with higher parity. The higher rates of caesarean delivery among low parity women strongly suggest the misuse of this surgical procedure by obstetricians, presumably for lucrative reasons.

As regarding to infant characteristicsthere were no statistical significant difference between both groups in infant sex. Male infants were 11(37.3%) and 8 (53.3%) in vaginal and cesarean group respectively and female were 4(26.7%) and 7(46.7%) in both groups respectively (p 0.256).

As regarding to gestational age (GA); the mean GA was 39.6+1.4 range (37-42 wk) in vaginal group and 38.8+1.4(range 36-41wk) with no statistical significant difference between both groups (p=0.126).

This is in contrary with ***Atsuko et al. (2010***) who found that cesarean section deliveries had significantly shorter gestational age and relatively higher than vaginal deliveries.

Thisis because of in that study the number of vaginal deliveries was 105 and that of cesarean deliveries 21 however in the present study equal number of both types of delivery was taken.

As regarding fetal weight (kg), the mean fetal weight was 3.2+0.6kg (range2.5-4.5) in vaginal group and 3.1+0.7kg (range 2-4) in cesarean group with no statistical significant difference between both groups (p =0.650). this is in contrary with ***Atsuko et al. (2010***) who found that neonates delivered vaginally were significantly larger than those delivered by cesarean section.

As regarding to placental weight (g) the mean placental weight was 673.3+234.4 (range 400-1100g) in vaginal delivery and was 666.7+244 (range 400-1100 g in cesarean group with no statistical significant difference in both groups (p= 0.940).

In the present study the mean cord blood volumewas 65.5+14.1 ml (range 45.0-90.0) Vs 78.5+16.9 ml (range 55.0-105.0) in vaginal and cesarean groups respectively with significantly higher Blood Volume in cesarean group (95% CI -24-1.0 & p = 0.034). These findings agree with the result of ***Yamada et al. (2000)*** who compared 29 cesarean deliveries with 126 vaginal deliveries andsuggested that CB volume following cesarean sections after placental expulsion was greater (103.9 vs. 84.2 mL), similer to the study of ***Tulika et al (2011)*** who compared173 vaginal deliveries with 327 cesarean deliveries, suggested that CB volume was significantly higher in cesarean deliveries (P< 0.01).

However, these findings were in contrary with the result of ***Atsuko et al (2012)*** who included 916 CB units 847from vaginal and 69 from deliveries cesarean andfound that almost the same amount of CB was obtained from vaginal and cesarean section deliveries (80.9 vs. 79.1 mL, respectively; p = 0.557) and the mean CB volume (80.7 ± 23.7 mL) was rather higher than that in previous studies. Thus variations of CB volume may be caused by the experience of the obstetric staff collecting CB, or the position of the umbilical cord and the infant prior to clamping and placing the newborn on the maternal abdomen after delivery could increase the volumeof umbilical CB ***(Pafumi et al., 2001).***

As regarding to umbilical cord length there was no statistical significant difference between vaginal and cesarean groups.

In the present study there was no statistical significant difference between vaginal and cesarean groups regarding total nucleated cells (TNC), this result in contrary with that of ***Atsuko et al. (2012)*** study in which a significantly higher TNC was observed in the vaginal, primiparous, and meconium-stained amniotic fluid deliveries when compared with cesarean section, multiparous, andnonstained amniotic fluid deliveries, respectively.

Also ***Shu-Hui et al., (2012)*** study which included 1549 UCB units, 75.27% from vaginal 24.73 from cesarean sectiondemonestrated that UCB of infants with vaginal delivery had more TNC but less cord blood volume.

This may be due to large scale study of CB units in both of these studies than in present study.

In the present study CD34+ count was in significantly higher among cesarean group than among vaginal group, this is in agreement with ***Tulika et al. (2011)*** who included 500 CB units 173 from

vaginal and 327 from cesarean deliveries and found that CD34+ cell count was higher in cesarean deliveries than in vaginal deliveries.

This is in contrary to ***Atsuko et al. (2010) who*** found that the number of cord blood units collected from cesarean deliveries was extremely low, especially regarding CD34+.

This is because neonates delivered vaginally were significantly larger than those delivered by cesarean section in the last study.

As regarding to CD45, there was no statistical significant difference between both groups.

CD45+ & CD34+count was insignificantly lower among cesarean group than among vaginal group.

This finding was in contrarywith ***Sahar et al (2011)*** who didnot support any effect of mode of delivery on CD45+/CD34+ in neonates.

This is because these (25) infants were preterm and all fullterm (25) neonates in their study were delivered vaginally except for one.

There was no statistical significant difference between vaginal and cesarean groups regarding to viability***. Sara, (2015)*** who included 206 CB units, found thathigher viability was associated with a bigger baby (p=0.033) and high number of birth order (p=0.044), this is may be due to delivery of all women in this study vaginal delivery.

There was significant positive correlationbetween cord blood volume and cord length (r 0.966 vs 0.964), infant weight (r 0.824 vs 0.980), placental weight (0.822 vs 0.976) as well as CD34 (r 0, 933 vs 0.976), in vaginal and cesarean groups (p <0.001). These results are in agreement withthe Study of ***Raquel et al., (2007)*** who reported that there was a positive correlation between volume of collected umbilical cord blood and newborn weight as well as a positive correlation between newborn weight with CD34+ cells and TNC.

Also ***Tulika et al (2011)*** stated that there was a positive correlation between volume of collected UCB and higher birth weight of the baby, (p < 0.01) which can thus result in an increase in the absolute number of CD34+ cells.

There were significant positive correlation between cord blood volume and CD45+ & CD34+ (p 0.050 & r -0.513) as well as CD45- & CD34+ (p<0.001 & r0.963) in cesarean group.

No significant correlation between cord length and other variables (maternal age, infant GA, infant weight, placental weight, TNCcount, CD34+, CD45, CD34+ & CD45+, CD45- & CD34+ and viability) in vaginal and cesarean groups.

Thisagrees with ***Shu-Hui (2012)*** who found that there were no significant as sociation between the length of the umbilical cord and each of the UCB clinical laboratory variables.

In the present study there was significant negative correlation between maternal age and CD45 in vaginal group. This finding agree with ***Sara (2015)*** study who foundthe inversely proportional relationship between maternal age and $CD45^{+}$ cell count Meanwhile, some previous studies reported that maternal age affected neither TNC or CD34+ cells ***(Omori et al., 2008)*** and that there was no loss of hematopoietic potential in babies delivered by women between the ages of 35 and 40 years ***(Ballen et al., 2001).***

Also ***Shu-Hui (2012)*** found that there was no significant association between maternal ageand UCB variables.

However, the study of ***Atsuko et al (2012)*** found that the TNC from primiparae aged 30–34 years was significantly higher than that from primiparae aged 20–24 years. This result was supported by a previous study of ***Omori et al., (2008) and Mohyeddin et al. (2004)*** who suggested that TNC significantly increases in women more than 25 years of age and with one or two parities.

In the present study no significant correlationwas foundbetween gestational age and other variables.

Thisagree with ***Ayad et al (2014***) who included 124 CB units andfound no significant effect of gestational age on TNC or CD34 cell count of the collected UCB.

However this is in contrary with ***Shu-Hui et al (2012)*** who found that the length of the gestational period affected the CD34+ cell number and UCB volume but not the TNC number and that the longer the gestational period, the less the CD34+ cell number and the UCB volume. Meanwhile ***Mancinelli et al., (2006)*** indicated that longer gestational period (>39 week) increased the total number of CD34+ cells. This is because gestational age in both groups show great similarity with no statistical significant difference.

In the present study as regarding correlation between infant weight & placental weight and other variables in vaginal and cesarean groups, there were significant positive correlations between infant weight as well as CD34 in both groups.

There were significant positive correlations between placental weight and infant weight as well as CD45- & Cd34+in cesarean group. This agrees with ***Tulika et al (2011)*** who found that the mean of the CB volume and CD34+ cells concentration were significantly higher in heavier birth weight of the baby than in normal birth weight of the baby (p < 0.01).

Also in accordance with our findings, ***Shu-Hui et al., (2010)*** who found that placental weight was positivelyassociated with all UCB clinical laboratory variables. Birth weight was positively correlated with TNC and volume but not with CD34+ cell number.

Sara, 2015found that the birth weight was associated positively with umbilical cord blood volume, placental weight, $CD34^+$, and TNC counts. This significant observation may be largely due to the birth weight, which could be directly affected by placental volume.

In the present study there was significant negative correlation between placental weight and CD45+in the same group. Inspite of the scanty published information on the $CD45^+$ leukocyte antigen count in cord blood, we studied the association between $CD45^+$ with maternal and neonatal factors, TNCs, umbilical cord blood volume, and $CD34^+$ count. Furthermore, $CD45^+$ count may be used as a predictive factor for the quality of T cell recovery and consequently for the risk assessment of life-threatening infections. In addition, the antigen CD45 is one of the most important factors in the detection of $CD34^+$ by different qualitative and quantitative protocols the present study hypothesis was supported by ***Azouna et al (2011)*** whoincluded the antigen CD45 in their study panels.

As regarding to infant sex there were no statistical significant difference between male and female in both groups in relation to stem cell results. These findings agree with ***Tulik et al (2011)*** where baby sex has no effect on total volume of cord blood collection and CD34+ cells concentration.

In the present study as regarding to birth order there were no significant difference between primigravida and multigravida in vaginal & cesarean group in relation to stem cell results.

This agrees with ***Ayad et (2014)*** who found no significant correlation between birth order and UCB volume, TNC, and CD34 levels. In ***Lim et al (2000)*** study it was found thatthe first neonates showed a significantly higher TNC than any other subsequent neonates because of a prolonged first stage of labor, which may occur in primiparae, is provably associated with an increase in TNC, granulocytes, CD34+ cells, and hematopoietic progenitor cells. This indicates that stress and infection may increase neutrophil numbers in TNC.

In conclusion:

Total volume of cord blood and CD34+ cells concentration were positively correlated with cesarean delivery and higher birth weight of the baby. Baby sex have no effect on cord blood volume and CD34+ cells concentration.

We hope that our observations and the observations of the previous investigations that were confirmed by the current study will help cord blood banking programs to establish strategies based on feasible circumstances in the selection of UCB that will require fewer resources and simultaneously maintain agood quality of the UCB units that are most likely to be employed for transplantation in the future.

However Further studies will be required after increasing the number of CB units collected by cesarean section delivery in order to overcome the limitation of smale sample size in the present study.

Recommendation

1- Further studies will be required after increasing the number of CB units collected by cesarean section delivery in order to overcome these limitations.

2- Indication of mode of delivery should follow guidelines protocols for the benefit of mother and neonat as followed in our hospital.

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