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Umbilical Cord Blood Stem Cells Bank, Collection of UCSCs during Delivery

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Abstract: ***Background: Until now, blood that remained in the umbilical cord and placenta after delivery was routinely discarded as garbage. Objective: Evaluation of different factors affecting UCSCs collection and cryopreservation to be used further in transplantation for different therapies. Methods: After approval of Ethics Committee and patient consent, randomly selected 40 Umbilical cord blood samples were drawn from 40 healthy pregnant women during delivery. Mononuclear cell separation was done. CD34$^+$stem cells were obtained using MACS stem cells separator . Cord blood samples were cryopreserved. HLA genotyping was done for screening 8 of the samples by NGS after DNA extraction. Results: UCB stem cells collection was affected by birth weight, sex of new born, volume of fresh blood and length of UC. The percentage of CD34$^+$stem cells viability after thawing was not affected throughout cryopreservation period. HLA genotyping was a good tool by NGS. Conclusions: UCB is a cheap, easily and rich source of hematopoietic stem cells. A chance that is given to man once in life time and particularly at the moment of birth, So SCs are the seeds that deserve to be banked not disposed as garbage. UCSC Collection is non-invasive, painless, and poses no risk. NGS over has a great value in the context of HSCT for cord blood banking which was suggested as a complementary step.***

1. Introduction

Stem cells are one of the most fascinating areas of biology today. Research on stem cells is advancing knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. This promising area of science is also leading scientists to investigate the possibility of cell-based therapies to treat disease, which is often referred to as regenerative or regenerative medicine (**National Institutes of Health (2011)** which offers identifying safe and ethically acceptable stem cell source for the development of therapeutic strategies (**http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2593758/ (2008)**). Stem cells are cells that have the capacity of self renewal by dividing and to develop into more mature, specialized cells. Stem cells can be unipotent, multipotent, pluripotent or totipotent, depending on the number of cell types to which they can give rise **(https://www.nature.com/subjects/stem-cells).** As the newborn is delivered, and the umbilical cord is divided, blood can be collected from the segment of cord still attached to the placenta. Stem cells retrieved from the blood in the remaining segment of the umbilical cord and placenta are known as 'umbilical cord blood (UCB) stem cells. This blood which is of no use to the mother or the baby and has been treated as a medical waste for centuries; also it is a rich source of stem cells. UCB stem cells are unique and have many promising uses for the future. As these cells are naïve, on allogeneic transplantation, they produce an attenuated donor derived immune response and thus have a lower incidence of graft versus host reaction when compared to other sources of stem cells (bone marrow or peripheralcells). Unlike other sources, these can also be transplanted even without an identical HLA match. The collection procedure is easy and without any risk to the donor (mother or baby) (**Ballen*et al*.,2008**). Umbilical cord blood (UCB) contains hematopoietic stem cells **(Ingrid Gessink *et al*. (2008).** These cells are characterized by an expression of CD34 antigen, which are also called CD34 cells **(Fasouliotis and Schenker (2000)**. Three types of banks have emerged for the collection and storage of umbilical cord blood: public, private banks and hybrid ones **(UCB banking (2015**).NGS technology has demonstrated the capacity to sequence DNA at unprecedented speed, thereby enabling previously unimaginable scientific achievements and novel biological applications. But, the massive data produced by NGS also presents a significant challenge for data storage, analyses, and management solutions. Advanced bioinformatics tools are essential for the successful application of NGS technology **(Jun Zhang and Rod Chiodini *et al*., 2011)**.

2. Subjects and Methods

The study was a Prospective Observational Randomized Laboratory Study carried out at Animal Biotechnology Department, GEBRI, Sadat City University, Obstetrics and Gynecology Department, Menoufia University Hospital & Research Centers of Excellence, Tanta Educational Hospital, from December 2015 & ended at January 2017. 40 Umbilical cord blood samples were drawn from 40 pregnant women (selected randomly, 10 Spontaneous vaginal delivery, 30 Caesarian section delivery), a written informed consent was taken from all of patients after explanation the nature of the study before delivery, The files of the included 40 women were revised from Obstetrics and Gynecology department, Faculty of Medicine, Menoufia University Hospital and the selected women were: Educated healthy women with age range between 22 to 35 years old, Full term pregnancy (37- 40 weeks) and women suffer from any chronic diseases and taking any medications were excluded.*All the data were gained in the presence of Obs. &Gyn. Senior Resident. UCB samples (n = 40) were aspirated from the umbilical cord vein after 3-5 minutes of delivery. CB collected by gravity into a tube system containing 5 mL of citrate phosphate dextrose anticoagulant. All UCB samples were processed within 6h after deliveries. Throughout this procedure, the delivery of the baby and the

timing of umbilical cord clamping was not affected in anyway. In the same time length of UC was measured, placenta was weighed and Apgar scores of the delivered new born were calculated. CB samples were transported in a cooled environment (cooler) (4-25°C).Between collection and transportation, samples were maintained at refrigerated temperatures (2-8°C) without agitaion. Mononuclear cells separation was done by Density gradient; Ficoll-paque with precautions: As a start Cord Blood sample, PBS and Ficoll-Paque were warmed to room temperature. Fresh human cord blood samples treated with citrate phosphate dextrose anti-coagulant were diluted with 1-3 volumes phosphate buffer saline solution (PBS) to reduce the density of the blood making layering over Ficoll easier and reduce the cell concentration preventing MNCs from becoming trapped as the erythrocytes aggregate and fall to the bottom of the tube.2. 15-30 ml of diluted cell suspension were carefully layered over 15 ml Ficoll-paque in a 50 ml Falcon tube and centrifuged at 2500 rpm or 400 xg for 20 minutes at 20°C in a swinging-bucket rotor with brake off to accelerate the density gradient separation.The upper layer (buffy coat layer of WBCs was at the interface between the sample and the medium) was aspirated off using a sterile Pasteur pipette, leaving the mononuclear cell layer (lymphocytes, thrombocytes and monocytes) undisturbed at the interphase. Carefully the mononuclear cell layer (Buffy coat) was transferred into a new 50 ml Falcon tube. The Falcon tube was filled with rinsing buffer, mixed well and centrifuged at 1200 rpm or 300 xg for 10 minutes at 20°C then the supernatant was carefully removed completely..The pellet was resuspended in 50 ml of buffer and centrifuged at 1000 rpm for 10-15 minutes at 20°C to increase the purity of the cells then the supernatant was carefully removed completely. The pellet might be resuspended again for more washing (if there was blood remains) in 50 ml of buffer and centrifuged at 1000 rpm for 10-15 minutes at 20°C then the supernatant was carefully removed completely. Viability test and cell counting was done by mixing 50 ml of cell suspension with 10 ml of 0.4 % Trypan blue stain in a cryo-tube, mix by pipetting up and down, incubate mixture for less than three minutes at room temperature. If cells are counted after approximately five minutes, viability will be inaccurate due to cell death, then put 10 of the mixture beneath the cover slip on a hemacytometer. Examine under the microscope. The viable cells didn't take up the dye and the unviable cells stained dark blue. The hemacytometer and cover slide were rinsed with 70% alcohol, and wiped dry. The pellet was resuspended in a final volume of 300 µl / 108 total cells then magnetic labeling was proceeded.

Immuno-magnetic labeling and separation of $CD34^{\pm}$ Progenitor Stem Cells by MACS stem cells separator.100µl FCR Blocking Reagent per 10^8 total cells was added to the cell suspension. 100 µl $CD34^+$ Micro Beads per 10^8 total cells was added for labelling cells, then mixed well and incubated for 30 minutes in the refrigerator at 2- 8°C. Cells was washed carefully by adding 1-2 ml buffer per 108 cells and centrifuged at 300 xg for 10 minutes. Supernatant aspirated completely then it resuspended up to 10^8cells in 300 µl of MACS separation buffer Magnetic separation with auto MACS® separator: The instrument was prepared with columns. Tube containing the sample was applied and tubes for selecting labeled and unlabeled cell fractions were provided. Positive selection for $CD34^+$ fraction and negative selection for $CD34^-$ fraction were chosen.

Cryopreservation: Cell freezing:$1x10^6$viable cells resuspended in 1.5 ml cryoprotectant medium (DMSO) at a cryoprotectant tube, slowly freeze with 4°C for 10 min as a start temperature. Next transfer to -20°C freezer then transfer to -80°C for more than 6 months. The samples were then transferred to the gaseous phase of a liquid nitrogen Dewar. Samples were frozen for a minimum 14 days before thawing, Cell thawing: Cryoprotectant vials of cells transferred as fast as possible to 37°C water bath, cells thawed within 3-5 min. Cells are centrifuged at 2500 rpm for 10 min. at 4°C then pellet resuspended in 25 ml DMEM growth medium supplemented with 20% fetal calf serum and seeded cell culture flasks with vented caps. The sample was then assessed for cell recoveries and viability. In some cases the samples were washed post-thaw in order to remove excess DMSO.Viability Test and Cell counting was done by heamocytometer.

Enrichment (Proliferation) for CD34 samples invitro:Culture of isolated CD34+ hematopoietic progenitor cells were performed in 75 cm3 cell culture flask with vented cap (Nunc A/S, Roskilde, Denmark) in DMEM supplemented with 10% FCS inactivated at 56°C for 30 min. Cells were incubated at 37°C in a 95% humidified atmosphere of 5% CO2 incubator. The growth medium was renewed every three days for two weeks. Investigation of cell viability and contamination occurrence was done under an inverted microscope. The old medium was aspirated off using a sterile pipette and 8 ml of pre-warmed trypsin (1:250) at 37°C was added to each flask and incubated at 37°C for 10 min allowing trypsin to work. Cell culture was examined under an inverted microscope; if cells were detached, the action of trypsin was deactivated by adding equal amounts of growth medium supplemented with 10% FCS.

A step toward CB banking, a genetic signature profile can be done for 8 cord blood samples for future use in transplantation therapies for HLA genomatching by NGS.DNA Extraction from cord blood (CD34) QIAamp®:40 µl QIAGEN Protease (or proteinase K) was pipetted into the bottom of a 1.5 ml microcentrifuge tube.400 µl of the CB was added to the microcentrifuge tube. It is important to ensure proper mixing with the enzyme.400 µl Buffer AL was added to the sample. Mix by pulse-vortexing for 15 s. to ensure efficient lysis, Incubate at 56°C for 10 min. The microcentrifuge tube was centrifuged to remove drops from the inside of the lid.400 µl ethanol (96-100%) was added to the sample and mixed by pulse-vortexing for 15 s. After mixing, the microcentrifuge tube was centrifuged to remove drops from the inside of the lid. The mixture from step 5 was applied carefully to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. The cap was closed, and centrifuged at 6000 xg (8000 rpm) for 1 min. at room temperature. - If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Mini spin column is empty. Carefully the QIAamp Mini spin column was opened and 500 µl Buffer AW1 was added without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1

min.The QIAamp Mini spin column was opened carefully and 500 µl Buffer AW2 was added without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. The QIAamp Mini spin column was placed in a clean 1.5 ml microcentrifuge tube, discard the collection tube containing the filtrate. The QIAamp Mini spin column was opened carefully and 200 µl Buffer AE or distilled water was added. Incubate at room temperature (15-25°C) for 1 min. to increase DNA yield up to 15%, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

Calculation of DNA purity on Nanodrop Spectrophotometer: The DNA concentration and purity were assessed by measuring the UV absorption of the sample at 260 and 280 nm using 2 µL of the DNA solution on a Nano Drop ND-1000 (Nano Drop, Wilmington, DE, USA) spectrophotometer. DNA was then diluted to a final concentration of 0.1 µg/µL using DNA Hydration Solution.*Extracted DNA can be stored at -80ºC.Using next generation sequencing illumina MiSeqDx, all eight extracted DNA samples can be genetically tested to be free from any genetic diseases that affect the blood and immune system. It can be used further as a hope in the umbilical cord blood SCs banks for HLA genotyping in donation, transplantation therapies and regenerative medicine.

Statistical analysis

Results were statistically analyzed by statistical package SPSS version 20. Two types of statistics were done.Descriptive: were used to compare the MNC and $CD34^+$ cell counts, viability, and cell count recovery e.g. Percentage (%), mean and standard deviation (SD).Analytical: Mann-Whitney test: it is a nonparametric test to compare two groups of non-normally distributed data Fisher's exact test: it is used to compare between two groups regarding one qualitative when the count of any of the expected cells less than 5.Student's t-test: it is used for a normally distributed quantitative variable.NGS data analysis: by (version 1.7/1.8, Omixon) and NGSengine (version 1.3/1.6/ 1.9/2.1, GenDX, Utrecht, the Netherlands).

3. Results

The factors were used as a nucleus for studying the factors affecting umbilical cord blood stem cells collection and preservation so cord blood bank quality can be achieved. The demographic data of 40 studied women as a source of UCSC are illustrated in table(1)

Table 1: Demographic data of 40 studied women as a source of UCSC in the study

	No.	Mean ± SD	Median (Range)	P Value
Maternal age (years)				
22 – 27	23	10.17 ± 0.14	10.32 (9.95 – 10.4)	0.988
28 – 35	17	10.17 ± 0.11	10.18 (9.94 – 10.4)	
Parity				
3	40	10.36 ± 0.12	10.19 (9.95 – 10.4)	0.484
Gestational age at delivery (weeks)				
37 – 40	40	10.17 ± 0.12	10.21 (9.94 – 10.4)	0.051
Birth (Neonatal) weight (gm)				
2900–4000	40	10.20 ± 0.11	10.37 (9.98 – 10.4)	0.023
Sex of infant				
Male	12	10.16 ± 0.12	10.16 (9.94 – 10.4)	0.045
Female	28	10.34 ± 0.09	10.34 (9.98 – 10.4)	
Mode of delivery				
NVD	10	10.17 ± 0.09	10.24 (9.95 – 10.3)	0.738
CS	30	10.17 ± 0.12	10.15 (9.94 – 10.4)	
CB Collection method				
In utero	9	10.16 ± 0.10	10.18 (9.95 – 10.3)	0.037
Ex utero	31	10.17 ± 0.12	10.19 (9.94 – 10.4)	
Hours between collection & processing (hrs)				
4	3	10.20 ± 0.15	10.25 (9.98 – 10.3)	0.854
5	14	10.17 ± 0.10	10.19 (9.95 – 10.3)	
6	23	10.17 ± 0.12	10.25 (9.94 – 10.4)	
Volume of fresh UCB (ml)				
80	22	10.3 ± 0.17	10. 24 (9.88-10.5)	0.034
>80	18	10.8 ± 0.16	10. 14 (9.52-10.5)	
Length of UC (cm) from placental insertion to collection site				
35- 50	27	10.40 ± 0.16	10.23 (9.24 – 10.4)	0.044
>50	13	10.28 ± 0.12	10.35 (9.98 – 10.4)	

P value < 0.05 is significant.

Table 2: Effect of transport temperature on MNC Count

Transport temperature (°C)	Number	Total UCB MNC yield x106(Median)	P value
4	32	1.86 ± 0.92	0.001
>4	8	2.55± 2.48	0.03

P value < 0.05 is significant.

Table 3: Effect of UCB volume on CD34 yield

Volume of UCB (ml)	Number	CD34x106 (Median)	P value
80	22	1.46 ± 0.86	0.02
>80	18	2.03± 2.40	0.002

P value < 0.05 is significant.

Table 4: Changes in numbers of cryopreserved cells, thawed cells, viable cells and viability % with time

	Median	Mean Rank	$\chi 2$ of Friedman test	P value
Number of Cryopreserved cells (x106 Cells)	10.18	5.58	85.124	<0.001
Number of Thawed cells (x106 Cells)	10.15	2.85		
Number of Viable cells (x106 cells)	9.87	4.95	70.566	<0.001
Viability %	92.70 %	4.76	87.246	<0.001

P value < 0.05 is significant

Temperature during transport of CB samples was considered an important factor affecting MNC yield. The best temperature for MNC recovery was 4 °C (P=0.001) as shown in table (2). However, recovery was good for CB samples at temperature more than 4°C.By increasing the UCB volume (>80 ml), CD34 yield increased, Also There was a clear significant for CD34 yield in volume 80 ml (P=0.02) as shown in table (3). There was also a positive relationship between UC length and volume of the collected blood. Table (4) showed that there was a significant change in the number of the thawed cells with time with viability 92.70% (P<0.001)

Table 5: Absorbance level of extracted DNA from 8 samples of UCB

Number of samples	Mean 260/280 ratio± SD	260/280 Range	P Value
8	1.84±0.09	1.59-2.04	0.001

P value < 0.05 is significant

Table 6: Number of total NGS typing per locus

Locus	Total NGS typing
A	8
B	8
C	8
DRB1	6
DRB3	7
DRB4	3
DRB5	5
DQB1	5
DPB1	6

Table 7: Sequencing depth and coverage in the eight samples with discrepant results in HLA interpretation by the Omixon Explore software

	Exons covered	Depth range	Exon 1	Exon 2	Exon 3	Exon 4	Exon 5	Exon 6	Exon 7
All samples HLA-DQB1	5	3–279	37–305	3–105	51–446	40–307	155–413	na	na
All samples HLA-A	7	16–322	4–206	5–239	4–305	4–463	12–426	1–389	1–366

By measurement of absorbance level of extracted DNA on spectrophotometer, P value was significant. Extracted DNA samples were pooled and sequenced using an Illumina MiSeqDx sequencer (V2; 2x250 bp -paired-end reads-; Illumina Inc, San Diego, CA). NGS data, Target by (version 1.7/1.8, Omixon) and NGS engine (version 1.3/1.6/ 1.9/2.1, GenDX, Utrecht, the Netherlands).The 2 genotyping programs used for analysis to safeguard against systematic analysis errors agreed in 94.8% (1of 7) of the best match allele calls up to the third field. Ambiguous results were most common at the DPB1 locus due to a lack of phasing between exons 2 and 3 or the unsequenced exon 1 (17% of alleles) and the DRB1 locus due to not sequencing exon 1 (3.8% of alleles). No ambiguities were detected among the other loci. Genotyping 8 samples using NGS, with a very high concordance rate with other HLA typing methods and low ambiguity rates for DRB1 and DPB1.

4. Discussion

Umbilical cord blood is increasingly used as an alternative source of hematopoietic stem cells in related and unrelated allogeneic transplantation in children and adolescents for almost two decades. The characterization of UCB units facilitates the understanding of factors affecting the quality and improvement of transplant outcomes **(Aroviita *et al.*, 2005)**.The optimal yield of mononuclear cells remains the most important aspect of UCB collection. The amount of fetal blood remaining in the placenta and the umbilical cord after clamping and dissection depends on several factors. The technique of UCB collection varies between different cord blood banks **(Gluckman *et al.*,1997).**The collection from the placenta in utero is easy and does not disturb the natural course of birth or the postpartum period. Multiple punctures or placenta manipulation are other means of increasing the amount of UCB collected. The amount of NC obtained with the placenta still in utero was higher than for collections ex utero, even though the volume collected was similar using both techniques. Because of the immune compromised state of UCB recipients, it is important to ensure the absence of possible pathogens in the graft. A decrease in bacterial contamination in the units collected after cesarean section, which is in keeping with the fact that 95% of the positive samples could be attributed to skin and vaginal flora. Maternal cells can be detected in UCB at a frequency of less than 1 in 10000 cells, no cases of GVHD secondary to engraftment of these cells has occurred to date, and no correlation between maternal cell content in the graft and incidence or severity of GVHD or graft rejection/ engraftment failure has been reported as yet. Some blood leakage from fetus/neonate to the mother occurs in up to 50% of births, usually during parturition, and, occasionally, clinically significant leakage occurs from mother to fetus **(Kurtzberg*et al.*,1996).Bertolini ´ *et al.* (1995)** reported that the magnitude of maternal contamination could be increased by UCB collection procedures, which try to collect optimal amounts of blood. Through genomic HLA typing of the 352 validated units no cases of maternal DNA contamination were detected, but in 27 samples examined using PCR amplification of 5 loci PM, maternal DNA sequences were identified in three out of the 27 UCB samples. 2 ng of DNA in the sample allowing to detect maternal DNA at levels ranging from 0.1 to 1%. Increasing the amount of DNA to 5 ng also raises the sensitivity of this approach, although specificity is decreased. Maternal DNA can be detected in a significant proportion of UCB units obtained after uncomplicated pregnancies, more often after normal labor and delivery than after cesarean sections, even though more units have to be tested to give a significant correlation between different groups. The mean volume collected, NC and CD34+ counts (84.6 6 23.6 ml, 0.90 60.37 3 109 and 2.46 6 2.72 3 106) using system were similar to those reported worldwide. Several centers have used separation methods for UCB prior to cryopreservation, and good recoveries have been obtained following HES sedimentation,**(Reboredo *et al.*, 2000)** 3% gelatin sedimentation **(Socie´ *et al.* (1994)** or Ficol density **gradients (Rubinstein *et al.* 1995)**..Apart from the methods described by Rubinstein ***et al*** **(Bertolini *et al.* (1996).(Sousa *et al.* (1997)** and **(Armitage *et al.* (1999)**the manipulations are performed in open systems, incompatible with normal blood banking procedures. When the method does not involve a closed bag system of separation, the risk of microbial contamination is increased.In comparison with other methods of sedimentation, HES 6% in NaCl does not require any laboratory preparation since it is commercially available and it can be used in a closed system, thus impeding possible microbial contamination during handling.

It is frequently used in surgery for volume replacement **(Sousa *et al*. (1997)** and is licensed in many countries for RCB removal from bone marrow to be used for transplantation **(Armitage *et al.* (1999)**.

UCB units can be routinely processed in a closed system that also achieves a significant reduction in storage needs and related costs, while maintaining quantity and quality of the progenitor cells. In the present study, density gradient separation method by ficoll paque or leucosep showed a good recovery of CD34 (2.03 ± 2.40) with P= 0.002 with increasing the volume of cord blood (10.8 ± 0.16) with P= 0.034.Since the first human CB transplant performed in 1988, CB banks (CBB) have been established worldwide for collection and cryopreservation of CB for allogeneic hematopoietic stem cells transplant **(Stehling , (1991)**. CB banking includes the following phases: (1) donor recruitment, consent, and medical evaluation of the donor; (2) CB collection; short-term storage and transportation; (3) processing, testing, cryopreservation, and storage; (4) release of CB unit to transplant center; (5) quality assurance according to FACT/NETCORD standards **(Walker ,1990)**.Data from previous studies showed that clinical outcome of cord blood transplantation depends on the number of nucleated cells transplanted, and most cord blood units do not contain enough stem cells for successful bone marrow reconstitution in adults **(Gluckman, *et al*.,2011]).**Engraftment happens by assessing the collection of UCB as well as volume reduction while maintaining sufficient progenitor cells. **Kurtzberg et al. (1996) and Gluckman*et al*.,(2011**) stated that the number of nucleated cells transfused per kilogram of body weight correlated with the rate of myeloid engraftment and that cell dose may be a more important indicator of engraftment than CD34+ cell or CFU-GM values.A number of factors have been described that may influence the quantification of UCB CD34 cells and that may account for the variations in the reported results. Delivery mode was no significant. Delivery mode type via caesarean sections allowed significant increase in collected units volumes as compared to vaginal deliveries **(Tiercy, and Roosnek (2002)** which was not supported by others **(Lee *et al*. (2007and** Marsh **,2012).**

The difference in both modes of delivery consisting of the higher neutrophil concentration of female infants. In the present study, There was no relation between delivery mode and CD34 cells (P= 0.738).Some reports have shown that cesarean delivery provides collection of a higher volume of blood increasing the absolute value of CD34+ cells **(Aroviita *et al*.,2005).**

It has also been shown that factors like newborn weight and sex as well as maternal age can affect the concentration of $CD34^+$ cells **(Yamada *et al*.,2000)**. In the present study, no connection was detected between mode of delivery andmaternal age, although there was a positive correlation of volume of collected UCB with NB weight, which can thus result in an increase in the absolute number of $CD34^+$ cells. Bigger babies were associated with higher yields of MNC according to other reports **(Gluckman *et al.*, 1997)** and this association was relevant when the total maternal population was considered. **Page *et al*. (2014)** stated that high-quality umbilical cord blood samples should be defined as those with a larger collection volume and high $CD34^+$ cell count. Neonatal gender was a significant factor for $CD34^+$ viability percentage.

The average volume of cord blood collected in the present study was approximately 82.2 ml. The average CD34+ cells count was 2.2×10^5cells per unit, with a viability of 95.2% UCB cryopreservation as unmanipulated blood results in high costs for large-scale UCB banking. Depleting cord blood stem cells of RBC avoids exposure to incompatible red cell antigens, minimizes the infusion of free hemoglobin and limits the volume and the amount of DMSO needed for cryopreservation. Reducing the volume of UCB units should also help maintain their high cell viability, as the 70% alcohol followed by an iodine bath. Smaller volume allows them to be frozen more uniformly and thawed more rapidly. The capacity of volume reduced UCB units to reconstitute hematopoiesis has been confirmed**(Rubinstein *et al*. ,1995)**. In the present study, there was a significant change in the number of the thawed cells with time with viability 92.70% (P<0.001). Next-generation sequencing is a feasible option for performing all HLA genotyping in a clinical laboratory that serves various transplant programs. The method is reproducible, cost effective, accurate, efficient, and provides more-complete sequencing information of the HLA gene. Successful HLA typing by NGS requires the combination and molding of various elements, including the sequencing platform, the sample preparation protocol, and the bioinformatics (to analyze data quality and the ability of the software to handle the complexity of the HLA genes) to provide accurate HLA typing.The 4 million-bp major histocompatibility complex region in which the HLA genes are located has the greatest density of markers associated with different diseases and **traits (Vinzenz Lange *et al*.,2014)**.Complete characterization of the HLA genes and the major histocompatibility complex will lead to better understanding of their roles in health and disease.

5. Conclusions

Umbilical cord blood is a cheap, easy and rich source of hematopoietic stem cells. A chance that is given to man once in life time and particularly at the moment of birth, so SCs are the seeds that deserve to be banked not disposed as garbage.UCSC Collection is non-invasive, painless, and poses no risk, HLA genotyping for genetic library is vital and NGS over has a great value in the context of HSCT for cord blood banking which was suggested as a complementary step.

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