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Comparative Study between Three Methods of Stem Cell Separation from Cord Blood

Akram Deghady, Mona Wagdy Ayad , Yasser Orief, Mona Abo Bakr El-Shabassy
The Clinical Pathology Department and Obstetrics and Gynacology Department#, Faculty of Medicine ,University of Alexandria, Egypt

ABSTRACT

Background: Stem cells can be found in places like bone marrow and fat tissue, but the youngest, most flexible stem cells in the body come from the umbilical cord Several studies have shown the simplicity of umbilical cord blood collection however, stem cell separation method together with other factors such as unit size, maternal factors such as number of previous pregnancies age of the mother can affect the stem cells harvested from cord blood.
Aim of the work: Was to compare the outcome of three different methods for cord blood stem cells separation.
Material and methods: The study was carried out on 60 samples of umbilical cord blood collected from El Shatby Maternity Hospital, Faculty of Medicine, University of Alexandria.in this study we divided the samples into 3groups each of 20cord blood sample and used them to evaluate three separation methods: density gradient separation using Ficoll-Paque, an automated processing and harvesting system using AXP autoxpress thermogenesis, and magnetic bead separation method using MACS Columns and MACS Separators.
Results: There was a significant increase in the absolute number and the percentage of CD34 positive cells /total number of mononuclear cells harvested by the magnetic beads method 190.0±87.6 cell/µl, (5.4±2.8 %) compared to those separated by AXP autoxpress Thermogenesis method 20.1±10.9 cell/µl (0.7±0.4%), and density gradient separation method 62.6±78.1 cell/µl. (1.8±1.9 % of total nucleated cells .2.7% to 12.2% (5.4±2.8) more efficient than the other two methods.
Conclusion: The magnetic bead separation method was proven to be the most efficient method for CD34 recovery among the 3 methods used.
Keywords: Stem Cells, Magnetic Beads, Cord Blood.

I. INTRODUCTION

Stem cells are the building blocks of the body, and have the ability to create organs, blood, tissue, and the immune system. Stem cells can be found in places like bone marrow and fat tissue, but the youngest, most flexible stem cells in the body come from the umbilical cord.[(1)]
Several studies have shown the simplicity of umbilical cord blood collection, in addition to the lack of risk for both the mother and the newborn, low risk of graft-versus-host disease and low risk of transmitting infectious-contagious diseases.

The total nucleated cell (TNC) count and the stem cell number recoverable from a single unit is a major limitation that is affected by unit size, maternal factors such as number of previous pregnancies age of the mother [(2)], and the stem cell separation method. These factors highlight the need to make processing as efficient as possible [(3)] to make cord blood banking a real option.

Many methods are currently available and the purpose of this study was to evaluate three of them density gradient separation using Ficoll-Paque [(4)],automated processing and harvesting system using autoxpress- platform (AXP) Thermogenesis , and magnetic bead separation system using MACS Columns and MACS Separators.

II. METHODS AND MATERIAL

This study was done on 60 samples of umbilical cord blood obtained from females undergoing delivery by

caesarean section (For logistical and reasons of homogeneity of treatment) in El-Shatby Maternity Hospital, Faculty of Medicine, University of Alexandria.Samples were collected after an informed and written consent was gained from the parents. The protocol followed, was reviewed and approved by the Ethical Committee of The Faculty of Medicine, University of Alexandria.

UCB units were processed within 24 hours of collection and only if the sample exceeded 50 ml.

Collection of the samples started post- partum, before the placenta was delivered. The cord was clamped and Collection bags used containing citrate phosphate dextrose adenine (CPD-A) anticoagulant and had a needle attached (Baxter PL146-CPDA-1-35 ml Deerfield IL, USA).This was spiked into the bottom of the cord allowing the blood to drain into the collection bag by gravity and natural movement. Once sample collection was complete, the blood was transported to the laboratory and stored at room temperature until processing was initiated. For each sample, analysis for complete blood count was done pre and post processing.

Samples were divided into three groups each one included 20 collected samples:

First group: Stem cells were separated using the density gradient centrifugation method using Ficoll –Paque (Sigma-Aldrich- Germany).

Second group: Stem cells were separated using MACs column and MACs magnetic separator.
Third group: Stem cells were separated using the AXP autoxpress apparatus.

Density gradient; Ficoll-Paque: [4]

The UCB was diluted 1 : 1 (v/v) with a phosphate buffered saline (PBS) solution pH 7.2 and was then layered over the Ficoll (7:3 v/v). The sample was centrifuged at 300×g, for 15 min. The buffy layer of WBCs at the interface between the sample and the medium, was collected using a sterile Pasteur pipette. The buffy coat was then washed with PBS re-suspended and examined for CD34 positive cells in a flowcytometer.

CD34 MicroBead stem cell separation using MACSR columns and MACS separator (Miltenyi Biotec Inc.GmbH, Bergisch Gladbach, Germany): [5] Anticoagulated cord blood mononuclear cells (CBMNCs) were isolated by density gradient centrifugation, using Ficoll-Paque.

Cell pellet was re-suspended in buffer and centrifuged at 200×g for 10−15 minutes at 20 °C. The supernatant was carefully aspirated, the washing step was repeated, and the CD34+ cells were magnetically labeled with CD34 Micro Beads. Then, the cell suspension was loaded onto a MACSR Column which was placed in the magnetic field of a MACS Separator. The magnetically labeled CD34+ cells were retained within the column. The unlabeled cells run through; this cell fraction was thus depleted of CD34+ cells.

After removing the column from the magnetic field, the magnetically retained CD34+ cells were eluted as the positively selected cell fraction and counted.

AXP autoxpress- Thermogenesis : (Thermogenesis Corp., Rancho Cordova, CA, USA) :[6]

Is an automated, functionally closed, sterile system that harvests the stem cell-rich buffy coat (BC) from umbilical cord blood, with reduction of the initial unit volume to a user determined final volume of 20 to 22 ml of buffy coat. The buffy coat fraction contains concentrated white blood cells, including the stem-cell-rich mononuclear cells (MNC) population, along with red blood cells (RBCs) and plasma. The AXP automates this volume reduction process. Cord blood was transferred from the cord blood collection bag to the processing set, which was placed in the AXP device and centrifuged. During centrifugation, blood was stratified into three components: RBCs, buffy coat, and plasma. The AXP device harvested those components into separate bags. The stem-cell-rich buffy coat component was collected into a freezing bag and transported for cell counting and flowcytometery examination of CD34 cells.

The cell counts were performed using the Sysmex XT Analyzer (Sysmex Corporation, Kobe, Japan).

Flow cytometric analysis for CD34 cells was carried out using a Becton Dickinson FACS Caliber machine (BD Biosciences San Jose, CA, USA) [7].

100µl of cell pellet was transferred to a polystyrene test tube.

20µl of CD34 PE (immunostep research, Salamanca, Spain) was added and mixed gently with a vortex mixture. The tubes were then incubated at room temperature, in the dark for 20 min. The cells were then washed on the BD FACS Lyse/Wash Assistant, after which the samples were run on the flow cytometer.

III. RESULT AND DISCUSSION

No significant difference was found between the sources of cord blood for each group regarding maternal age, weight, number of gravida, birth outcome gender or weight as shown in table I.

Table (I) : Personal data of the source subjects for umbilical cord blood used for stem cell separation according to the method of separation

Personal data	Magnetic bead separation system (n=20)		AXP autoxpress-Thermogenesis (n=20)		Density gradient separation using Ficoll Paque (n=20)		Significance
	No.	%	No.	%	No.	%	
Gender of the fetus							LR=1.915 P=0.384
Female	12	60.0	10	50.0	6	30.0	
Male	8	40.0	10	50.0	14	70.0	
Fetal weight (grams)							
Min-Max Mean±SD	2630-3850 3159.9±416.3		2750-3750 3168.4±278.5		2860-3600 3190.8±233.4		$^{KW}X^2$=0.343 P=0.842
Number of pregnancies							LR=3.75 P=0.153
1-2	20	100	14	70	14	70	
3-4	0	0	6	30	6	30	
Maternal Age (years)							$^{KW}X^2$=1.91 P=0.385
Min-Max	20.0-33.0		24.0-37.0		23.0-33.0		
Mean±SD	26.4±3.7		28.8±3.7		27.3±3.4		
Maternal Weight (Kg)							$^{KW}X^2$=3.409 P=0.182
Min-Max	57.8-101.0		76.8-98.1		68.0-91.7		
Mean±SD	80.9±13.3		85.2±7.1		78.5±7.2		

$^{KW}X^2$: Kruskal Wallis test LR: Likelihood ratio

There was no significant difference between the three groups of cord blood samples regarding the volume, the white blood cells count or the mononuclear cell count (table II).

Table (II): Hematologic parameters of the source subjects for umbilical cord blood used for stem cell separation according to the method of separation

Hematologic parameters	Magnetic bead separation system (n=20)	AXP autoxpress-Thermogenesis (n=20)	Density gradient separation using Ficoll Paque (n=20)	Significance

CB volume (ml)				$^{KW}X^2$=0.221 P=0.895
Min-Max	72.0-110.0	56.6-124.0	58.2-116.0	
Mean±SD	90.2±12.1	87.9±17.8	87.6±17.5	
Total WBC count (x1000)/µl				$^{KW}X^2$=2.273 P=0.321
Min-Max	4.2-12.6	3.4-13.1	2.5-7.7	
Mean±SD	6.6±2.5	5.9±2.8	5.3±1.7	
Mononuclear count (x1000)/µl				$^{KW}X^2$=5.546 P=0.062
Min-Max	2.7-7.4	1.9-9.8	0.9-4.6	
Mean±SD	3.8±1.4	3.4±2.3	3.0±1.0	

$^{KW}X^2$: Kruskal Wallis test LR: Likelihood ratio

In this study separation of stem cells by magnetic beads and the MACS magnetic separator proved to give significantly higher yield of CD34 positive cells compared to the other two methods (P<0.0001) as shown in table III.

Table (III): Results of Stem cells harvested from the umbilical cord blood samples according to the three methods used for stem cell separation

CD34	(1) Magnetic bead separation system (n=20)	(2)AXP autoxpress-Thermogenesis (n=20)	(3)Density gradient separation using Ficoll Paque (n=20)	Significance	Tamhane's post Hoc test
CD34 absolute/µl				$^{KW}X^2$=17.836 P<0.0001*	(1,2)* (1,3)*
Min-Max	100-400	0.1-34	1.1-271		
Mean±SD	190.0±87.6	20.1±10.9	62.6±78.1		
CD 34%				$^{KW}X^2$=17.155 P<0.0001*	(1,2)* (1,3)*
Min-Max	2.7-12.2	0.03-1.2	0.12-6.4		
Mean±SD	5.4±2.8	0.7±0.4	1.8±1.9		

$^{KW}X^2$: Kruskal Wallis test *significant at P≤0.05

IV. DISCUSSION

Cord blood is one of the important sources of stem cells that have many clinical applications, there is an increased need for optimising separation, whether to reduce volume to make storage more efficient or to increase stem cell yield for transplant. Factors such as processing time, viability of residual cells, and exposure to various reagents also must be considered [(1)].

Magnetic cell sorting has become a standard method for cell separation in many different fields. Numerous publications have demonstrated its use at a small to large scale; from abundant cells to rare cells with complex phenotypes. [(8)]

The results of this study suggest that the magnetic bead separation method gives a higher recovery of CD34+ cells and this could be promising for successful therapeutic use and clinical applications provided that the function and viability of the target cells is not affected during separation and selection [(9)]. A weak point in the study is that we did not test viability of the harvested stem cells.

Pierzchalski et al [10] reported that high cell numbers can be isolated by this method. Positive selection, by labeling the target cells, is the fastest and the most efficient way to isolate a cell subset with high purity and yield. But because separation is based on a single parameter (i.e., magnetization), this method is generally effective only for the isolation of a single cell population. Moreover, the purity of the recovered yield was variable in different studies [11, 12], but some authors suggested that the magnetic separator performance in terms of purity and recovery is either comparable with or superior to other technologies. [13]

Others reported that isolation of stem cells from blood or bone marrow by MACS was a powerful technology for manipulation of the hematopoietic system. [14]

The AXP separation method is preferred by many authors to manual methods because of better standardization and reproducibility and less influence from the operator. RBCs depletion is another goal of volume reduction as RBCs contained in the cord blood product negatively influence the function of the progenitor cells recovered after thawing [15].

Unfortunately, in our study this method has the lowest yield of CD34 positive cells. Other investigators reported a loss of up to 30% of neutrophils is may be the main cause of the decreased TNC recovery with the AXP system, and the CD34+ cell recovery was low for UCB units with a high TNC content [16].However, the method allows greater number throughput with fixed personnel numbers increasing the economy of the operation than the manual methods. [17]

Separation by Ficoll-Paque gradient centrifugation allows rapid and efficient isolation of mononuclear cells from human blood [18]. As in fact, this technique is the starting point for most studies of human lymphoid cells, it does not change either the phenotype or the function of the isolated mononuclear cell population, but the method is inefficient for large volumes and requires considerable technical expertise [19].

Excessive handling and exposure to the environment may result in bacterial contamination. Inclusion of unwanted components, including neutrophils and platelets, is also problematic when using manual buffy coat preparation [20, 21].

V. CONCLUSION

In conclusion, the magnetic bead separation method was proven to be the most efficient method for CD34 recovery among the 3 methods used.

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VII. REFERENCES

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