



Viability of Umbilical Cord Blood for Haemopoietic Stem Cell Transplantation

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAOO did the conceptualization, literature review and interpretation of results. Author TMA wrote the manuscript performed the statistical analysis and managed the literature search. Author POO did the sample collection, data collection and write the manuscript. Author OA searched the literature and data interpretation. Author EE searched the literature and read the manuscript. Author GNB did the conceptualization and read the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Umbilical cord blood (UCB) can be used for haematopoietic stem transplant (HSCT) since it contains a sufficient number of haematopoietic stem cells and progenitor cells. Viability of cord blood is an important parameter in assessing UCB quality as a viable HSCT graft source. We assessed

the viability of cord blood between the time of collection and processing, and find out the relationship between viability count and processing time.

Study Design: A cross-sectional study.

Place and Duration of the Study: The study was conducted in University of Benin Teaching Hospital (UBTH), Benin City, South-South, Nigeria; among 40 newborn babies delivered at 37-40 weeks.

Methodology: A total of forty umbilical cord blood specimens were collected from the placenta after delivery. Viability count was done with molecular exclusion dye (7-aminoactinomycin D) using flow cytometer. Mean, standard deviation and correlation study were done using SPSS version 21.

Results: The mean viability count was $90.0 \pm 9.57\%$ with a range of 60.0 - 98.2%. The study showed a negative correlation between viability and processing time ($r = -0.859$, $p=0.000$).

Conclusion: The study showed that the delayed in the processing of cord blood affected the viability count.

Keywords: Umbilical cord blood; viability count; processing time; stem cell transplant.

1. INTRODUCTION

Umbilical cord blood (UCB) transplant is a viable alternative to bone marrow transplant. This is useful in the management of a wide range of haemopoietic diseases, such as malignant and genetic disorders [1]. Cord blood has the following advantages over other sources of stem cells: simplicity of sample collection, complete absence of risks to mother and newborn, provision of a readily available graft for the recipients without suitable matched related or unrelated donors, reduced need for human leukocyte antigen (HLA) compatibility testing, low risk of severe host-graft reaction and low risk of transmitting infectious diseases [2,3,4]. Therefore, umbilical cord blood can be preserved as a source of stem cells instead of seeing it as a biological waste [5].

Since 1989 when the first UCB was used for the treatment of Fanconi's anaemia, more than 6,000 unrelated donor cord-blood transplants have been done in 150 locations worldwide [2,6]. With increased recognition that cord blood constitutes a viable source of stem cells, cord blood banks were established worldwide to provide a large number of high-quality cord blood units for transplant centres [3]. Successful engraftment depends mostly on dose and quality of cells in the UCB unit [7]. Cell viability is an important indicator of the quality of umbilical cord blood (UCB) units that can influence transplant outcome. Both exogenous factors (time from collection to processing, collected UCB volume) and endogenous factors (total nucleated cell count (TNCC), $CD34^+$ cell count) affect cell viability [8]. Some studies have shown that extension of the time from collection to processing of UCB units can reduce the quality

by decreasing cell viability. The number of stem cells that persist in each UCB unit is limited, rendering the maintenance of cell viability a critical issue in the bio-banking industry [8,9].

There is a scarcity of data on the use of cord blood in Nigeria as stem cell transplant is still at its early stage of development. This is the thrust of our study. This study was done to assess the viability of cord blood and the effect of delay in the processing of UCB; and to find out if there is any relationship between viability count and processing time.

2. METHODOLOGY

2.1 Subject Location

This is a cross-sectional study carried out in the departments of Haematology and Obstetrics at the University of Benin Teaching Hospital (UBTH), Benin City, Edo State South-South Nigeria. UBTH is a tertiary health institution with the only stem cell transplant centre in West Africa [10].

2.2 Study Population

We recruited 40 newborn babies delivered at term with normal birth weight from consented mothers between the ages of 18-40 years who booked at UBTH and had uneventful antenatal history. Pregnant women with the following conditions were excluded from the study: mothers with infectious diseases, complicated pregnancies, women less than 18 years of age and those above 40 years, and mothers with diseases such as haemoglobinopathies and hypertension.

2.3 Sample Collection and Storage

Cord blood collections were performed after the delivery of the foetus. The umbilical cord was clamped and ligated before cutting to separate the baby from the maternal placenta. Once the placenta was separated and delivered, it was placed on a sterile sheet. The umbilical cord was carefully cleaned with methylated spirit and a total of four millilitres (4 ml) of cord blood was collected from the umbilical cord vein.

Three and a half millilitres (3.5 ml) of cord blood was dispensed into commercially prepared ethylene di-amine tetra-acetic acid (EDTA) bottle. The sample was mixed gently but thoroughly to prevent cell lysis and ensure anticoagulation. Samples were stored at 4°C and were transported to Haematology Laboratory of Ladoke Akintola University Teaching Hospital (LAUTECH) Ogbomosho, Osun State where they were assayed for total nucleated cell, viability count and CD34+ cells, within 24 hours of collections. Samples were analyzed in batches.

2.4 Cell Counts

Nucleated cell count was determined using the Sysmex haematology autoanalyser (KX21N, Japan). EDTA anticoagulated samples were mixed continuously on a mixer until analysed. Samples were consecutively placed in the receiver of the autoanalyser, which aspirated 20 µL of blood from the sample. Cell count was done automatically by the machine and the result printed out. These parameters include total nucleated cells and other haematological parameters of cord blood.

2.5 Viability and CD34 Assay

The CD34⁺ cell count was assessed using flow cytometric method on Cyflow cube 6 (Sysmex Partec, Germany). Umbilical cord blood was added to monoclonal antibodies for CD34 with the fluorochrome PE and incubated for 15 minutes in the dark at room temperature. The dye, 7-aminoactinomycin D was added to the

mixture and incubated at room temperature for 10 minutes. Erythrocyte lysing agent was added to the mixture and incubated for 20 minutes at room temperature in the dark. The sample mixture was analyzed on Cyflow cube 6 and the events were displayed in a plot of sidelight scatter versus forwarding light scatter. Gating was set around CD34+ cells and orange fluorescence associated with these events was displayed as a single-parameter histogram of number.

2.6 Statistical Analysis

Data were presented in tables and analysed using the SPSS 21 (Statistical Package for Scientific Solutions) software (Amonk, Chicago, IL). Variables were normally distributed after testing with Kolmogorov-Smirnov test. Statistical differences between means were tested using the Student t-test for two groups and ANOVA for more than two groups. Associations between variables were determined using Pearson's correlation coefficient. The socio-demographic data was presented as simple percentages. The correlations between TNC and CD34⁺ cells, viability count and processing time were represented on graphs. P -values less than 0.05 was considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

A total of forty (40) umbilical cord blood (UCB) samples were collected. Thirty three (33) of these samples were stored and analysed within twenty four (24) hours. While the seven other samples were stored and analysed after twenty-four hours.

Table 1 showed the mean viability count of cells and processing time. The mean viability of the cells was 90.00 ± 9.55% with a range of 60.0 - 98.20% while the mean processing time was 9.60 ± 4.47 hours with a range of 5.00– 22.0 hours.

Table 1. Mean and range distribution of CD34+cells, TNC, viability count and processing time

Variables	Mean ± SD	Range
TNC(x10 ⁶ cells/ml)	11.14 ± 4.64	2.90 - 21.10
CD34+(x 10 ⁴ cells/ml)	3.89 ± 1.48	2.00 - 6.99
Viability (%)	90.0 ± 9.55	60.0 - 98.20
PT (hours)	9.60 ± 4.47	5.00– 22.0

TNC: total nucleated cell, CD: clusters of differentiation, PT: processing time

TNC count and CD34+ cells decrease with prolonged storage time (greater than 24 hours) and were statistically significant (p = 0.00). Similarly, viability count tends to decline progressively with increasing storage time (greater than 24 hours) and they were statistically significant (p = 0.02).

Table 3 shows the correlation between TNC, CD34+ cells, viability and processing time (PT). A strong positive correlation was found between TNC and CD34+ cells (r= 0.760, P = 0.000) as in

Fig. 2A weak but statistically significant positive correlation was found between TNC and viability of the cells (r=0.475, p=0.005), while TNC has a significant moderate negative correlation with processing time (r= -0.505; p=0.003). CD34+ cells had a weak positive correlation with viability (r=0.195, p = 0.278). There was a very weak negative correlation between CD34+ cells and PT which was not statistically significant (r = -0.197, p=0.272), while viability had a very good negative correlation with processing time (r = -0.859, p =0.000) as shown in Fig. 2.

Table 2A. Comparing the mean distribution of TNC with storage time

Storage time	N	Mean ± SD of TNC x10 ⁶ /ml	t-values	p
<24 hours	33	11.14 ± 4.64	4.81	0.001
>24 hours	7	2.60 ± 0.30		

Table 2B. Comparing the mean distribution of CD 34+ cells with storage time

Storage duration(hrs)	N	Mean ± SD of CD34+cells (x10 ⁴ cells/mL)	t values	P values
<24	33	1.89± 1.48	6.602	0.001
>24	7	0.15 ± 0.174		

Table 2C. Comparing the mean distribution of viability count with storage time

Storage duration	N	Mean ± SD (%)	t values	p values
< 24 hours	33	90.00 ± 9.55	12.28	0.016
>24 hours	7	44.70± 0.99		

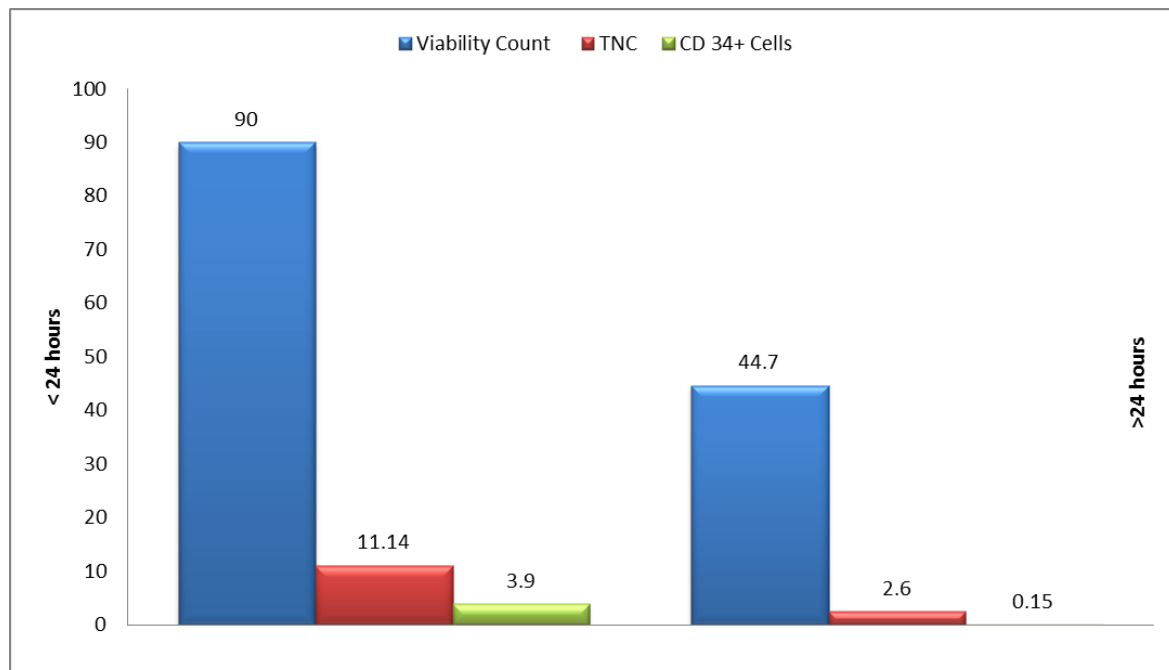


Fig. 1. Showing the mean value of TNC, CD34+ cells, and viability count with storage time (hours)

Table 3. Correlation between TNC, CD34+ cells, viability count and processing time(PT)

Variables		TNC	CD34 ⁺ cells	Viability	PT
TNC	r	1.00	0.760	0.475	-0.505
	P	-	0.0001	0.005	0.003
CD34 ⁺ cells	r	0.760	1.00	0.195	-0.197
	P	0.0001	-	0.278	0.109
Viability	r	0.475	0.195	1	-0.859
	P	0.005	0.278	-	0.0001
PT	r	-0.505	-0.197	-0.859	1
	P	0.003	0.272	0.0001	-

TNC: total nucleated cell, CD: clusters of differentiation, PT: processing time

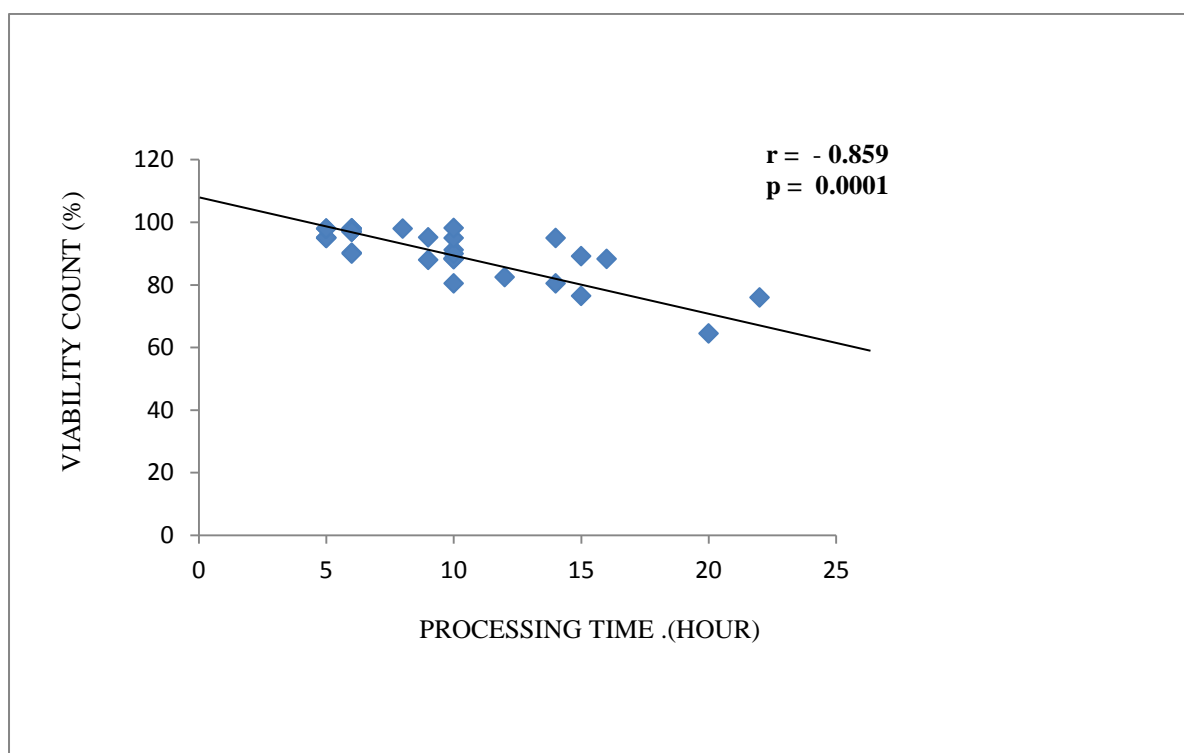


Fig. 2. Correlation graph of viability count versus processing time of cord blood

The above graph shows a negative correlation between viability count and PT.

3.2 Discussion

Umbilical cord blood (UCB) is a novel and unique source of transplantable stem cells that can be used as therapy for diseases indicative of haematopoietic stem cell transplantation (HSCT). The increased number of HSCTs over the past few years has demanded quality and safety improvements of stem cell processing and cryopreservation services. A cell viability count is one of the crucial parameters in assessing UCB quality as a viable HSCT graft source [11].

Viability count of umbilical cord blood cells in this study showed a mean value of 90%, range 60-98.2%. The value obtained was slightly lower than the values found in the previous study where a mean value of 98.4% was reported [12,13,14]. Low viability count in this study could be due to delayed processing time as a result of travelling from the collection centre to the processing centre (200 km). It was observed that the median processing time in this study was 10 hours. It has been reported that samples processed more than 10 hours from the collection have significantly lower values of viability count, TNC and CD34⁺ cells [15].

Processing time of the samples has a negative correlation with viability count, TNC and CD34+ cells. However, processing time versus CD34+ cells was not significantly affected, but viability count and TNC were affected. This is in agreement with the findings of other authors [16,17]. Isidro, et al. [18] reported reduced viability count in samples that were processed at a longer time. They also showed that CD34+ cells count did not differ significantly at the time of processing. Samples stored and processed after 24 hours tend to have low viability count and TNC count, the mean losses of TNC, CD34+ cells and viability count was $8.54 \times 10^6/\text{ml}$, $3.74 \times 10^4/\text{ml}$ and 45.5% respectively. Previous studies have shown that the numbers of TNC, CD34+ cells and viability count correlate well with outcome, thus most cord blood with a higher unit of TNC count and viability count are used for transplant in order to have a good outcome [19]. Several studies have shown that HSCT improves survival and disease-free survival rates when compared with conventional chemotherapy treatments, thus the rate of HSCT have increased significantly in developed countries [11]. It is important to know that cell recovery and viability rates are crucial parameters to assess UCB quality as a viable HSCT graft source.

It is therefore essential to maintain the viability of cells during transport and storage at a temperature of $1-8^\circ\text{C}$ and analysis done within 24 hours, as the likelihood of subjecting these tissues to extremes of temperature and prolong storage may decrease the cell count and viability [18,19]. Cord blood with low TNC, CD34+ cells and viability count are rejected by cord blood banks [19,20]. Interaction between temperature and storage time may influence the quality of haemopoietic stem cells.

4. CONCLUSION

The findings in this study shows that an increase in the time interval between collection and processing negatively affects the viability count of the UCB. It will be better if cord blood was processed within 24 hours of collections for maximum yield of stem cells and also to maintain above 90% viability count.

CONSENT

Informed consent was taking from the mothers.

ETHICAL APPROVAL

Ethical approval was obtained from the Ethical Committee of UBTH (Protocol number: ADM/E 22/A/VOL VII/1084) and utmost confidentiality was ensured in all the processes involved in the study from the questionnaire administration to sample processing and data analysis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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