International Journal of Research and Reports in Hematology

2(2): 144-157, 2019; Article no.IJR2H.52069

Assessment of CD34+ Cells and Total Nucleated Cells in Umbilical Cord Blood in a Tertiary Hospital South-south, Nigeria

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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 GNB did the conceptualization and read the manuscript. Author EE collected the cord blood and read the manuscript. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Dharmesh Chandra Sharma, ABTO (Associate Blood Transfusion Officer), Incharge Blood Bank, Component and Aphaeresis University, G. R. Medical College and J. A. Hospital, Gwalior, India.
Reviewers:
(1) Ramachandran Muthiah, Thoothukudi Medical College Hospital, India.
(2) Ota Fuchs, Institute of Hematology and Blood Transfusion, Czech Republic.
Complete Peer review History: http://www.sdiarticle4.com/review-history/52069

Original Research Article

Received 10 August 2019
Accepted 20 October 2019
Published 28 October 2019

ABSTRACT

Aim: Umbilical cord blood (UCB) contains sufficient number of haematopoietic stem cell and progenitor cells that can be used for autologous and allogeneic stem cell transplantation in children

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and adolescents. Our study assessed the CD34+ cells and total nucleated cells in umbilical cord blood stem cells in a tertiary institution in Nigeria.
Study Design: This is a cross-sectional study.
Place and Duration of Study: This study was conducted in University of Benin Teaching Hospital (UBTH), Benin City. Informed consent for UCB collection was obtained from healthy mothers with uncomplicated pregnancies, receiving care at the Department of Obstetrics between July and September, 2016.
Methodology: A total of forty umbilical cord bloods samples were collected from the placenta umbilical cord after delivery. CD34+ cells were enumerated using flow cytometer while haematology analyzer was used to assess total nucleated cell (TNC) count. Data was analysed using Statistical Package for Social Sciences (SPSS) version 21.
Results: CD 34+ cells count ranged between 2.0 - 6.99 x 10^4cells/ml with a mean value of 3.89 ± 1.48 x 10^4cells/ml (Recommended minimum value 2x10^5/kg). Mean value of TNC was 11.14 ± 4.47 x 10^6 cells/ml with a range of 4.80-21.10 x10^6 cells/ml (Recommended minimum value 2x 10^7 /kg). We observed a positive correlation between CD34+ cells and TNC count (r = 0.760, p=0.000). In addition, maternal parity showed a significant inverse relationship with TNC and CD34+ cells.
Conclusion: CD34+cells and TNC count of UCB obtained from placentae of babies delivered at the University of Benin Teaching Hospital are within the acceptable values for haematopoietic stem cell transplantation. This is in keeping with recommendations by the World Marrow Donor Association, which stated that a minimum of 2 x10^7 TNC/kg or 2 x10^5 CD34+ cells/kg of body weight of recipient.

Keywords: CD34+ cells; umbilical cord blood; haematopoietic stem cell; placentae.

1. INTRODUCTION

Haematopoietic stem cells (HSC) are cells capable of differentiating into several progenitor cells of different cell lines and have the capacity for extensive self-renewal or self-maintenance.[1] The progenitor cells being members of a cell population are capable of proliferating and differentiating into granulocyte, macrophages, erythrocyte and megakaryocytes [1] In haematopoietic stem cell transplantation (HSCT), stem cells are harvested from a donor and infused into a recipient to assume biological functions in the recipient's body [2,3] Cell engraftment and immune re-constitution in the recipient in the post-transplant period is integral to the success of any transplant. The functionality of the expectant differentiating cells is assessed using several modalities including modern single-cell technologies such as molecular profiling using single- cell transcriptomics and single-cell sorting using flow cytometry. These have the capacity to efficiently profile and characterize the phenotypic properties of individual cells, based on the differential expressions or cell-surface intracellular proteins. More recently mass cytometry is being used, although the automated cell counter may also be used [4,5,6]. Malignant and non-malignant disorders such as acute and chronic leukemia, lymphoma, solid tumours, immune deficiencies, inborn errors of metabolism and genetic diseases can be cured with HSCT [7]. Stem cells can be harvested from patient (autologous) or from related or unrelated (allogeneic) donors [8].

Sources of stem cells include bone marrow, peripheral blood progenitor cells and umbilical cord blood (UCB). HSC are harvested from the bone marrow or peripheral blood of a human leukocyte antigen (HLA)–matched sibling donor who can meet the stringent requirement of a 6/6 or 5/6 match with the patient's HLA loci [HLA-A, HLA-B, HLA- DRB1], while less stringent HLA 4/6 match is needed for UCB [8,9]. Unfortunately, only 30% of patients have HLA identical sibling donors. Identification of matching unrelated donors, particularly for minorities, can present an exceptional challenge. Studies have showed that recipient with HLA- mismatched cord blood transplantation (CBT) have a similar outcome with recipient who had transplant done with HLA matched BMT [10] and some other studies showed that those who had CB from HLA –identical siblings had low risk of acute and chronic GVHD compared to those who had BM from HLA –identical siblings [10]. Hence, transplantation of UCB represents the most recent strategy for expanding the potential donor pool while maintaining an acceptable level of treatment-related complications [11].

In 1989, Gluckman and colleagues published the first successful UCB transplantation using cord

blood from an HLA-identical sibling in a patient with Fanconi's anaemia [12].

The practice of UCB donation and banking is on the increase in many parts of the world [13]. Since 1989 when the first UCB was used for treatment, more than 6,000 unrelated donor cord-blood transplants have been done in 150 locations worldwide [13]. An average of about 100ml of blood can be harvested from a placenta for transplantation. In Nigeria, about 5 million children are born annually [14]. This amounts to about 500,000,000 ml of cord blood being wasted annually because they are not harvested. If UCB is put to use in Nigeria, it could become an important source of stem cell for HSCT [14,15].

Cord blood provides a readily available graft for the recipient without suitable matched related or unrelated donors [13].

Nigeria has the largest burden of sickle cell anaemia (SCA) in Africa [16,17]. At least 40 million Nigerians are carriers of sickle cell gene (AS) compared to 2 million in America. Over 150,000 Nigerians are born each year with SCA compared to 2,000 in America giving a prevalence of 3% in Nigeria [16,18]. Sickle cell anaemia, a genetic disease, and the only curative option for Hbss remains hematopoietic stem cell transplantation [18,19,20,21,22]. The two successful allogeneic HSCT done in Nigeria were from HLA matched sibling donors in 2011and 2013 respectively [18]. Although, these were done using bone marrow blood stem cells, however, it is hoped that as the program advances, the use of UCB for transplantation would play an integral part in successful outcomes of transplantation.

The engraftment, treatment related mortality and survival of recipients of UCBT are associated with the number of total nucleated cells (TNC) and the dose of CD 34 positive (+) cells. The World Marrow Donor Association recommends a minimum of 2 $x10^7$ TNC/kg or 2 $x10^5$ CD34+ cells/kg of body weight of recipient with viability of the nucleated cells greater than 75% [23,24] .

Previous studies on haematological parameters, showed that Caucasian values of total white blood cells count are higher compared to the black race, [25] hence it will be of great importance to determine the cord blood TNC count of Nigerian babies.

Our study determined the yield of CD34+ cells and total nucleated cells in umbilical cord. More so, we were able to establish the association between total nucleated cells and CD34+ cells in cord blood and other maternal parameters.

2. MATERIALS AND METHODS

2.1 Subject Location

This cross-sectional study was carried out at the University of Benin Teaching Hospital (UBTH), Benin City between July and September, 2016. This hospital serves as a regional referral centre for the surrounding states of Delta, Kogi, Ondo and other states like Anambra**.** Moreover, UBTH also has the only stem cell transplant centre in Nigeria, East, West, and Central Africa [18].

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 pregnant women 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) 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 4ºC 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 20µL 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 CD34/PE and was 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 side light scatter versus forward 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 was presented in tables and analysed using the SPSS Version 21(Statistical Package for Scientific Solutions) software. Statistical differences between means were tested using the student t-test and ANOVA. 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 were considered significant.

3. 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. Their results were compared with maternal and neonatal factors.

Demographic data, including maternal age, gestation age, gender, parity, placental weight and birth weight were documented and analysed. The laboratory data analysed included, total nucleated cell (TNC), CD34+ cells, viability count and processing time (PT).

Table 1A and 1B shows the mean, range and frequency of distribution of maternal age, parity, gender, mode of delivery, placental weight, birth weight and gestational age. The mean maternal age was 32 years with a range of 23 - 40 years. One (3%) of the mothers was <25years, while twenty five (75.8%) were between the ages of 25 – 35 years and seven (21.2%) were over 35 years old. Five (15.2%) of the mothers were primiparous, eleven (33.3%) were multiparous and 17 (51.5%) of them were grand-multiparous women.

The mean gestational age was 38.84 weeks with a range of 37-41 weeks, twenty (60.6%) of the women had a gestational age between 37-39 weeks, while thirteen (39.4%) were greater than 39 weeks. Eleven babies (33.3%) were delivered via caesarean section and twenty two (66.7%) by spontaneous vaginal delivery. The mean placenta weight was 548.48 grams with a range of 400-650grams. Five (15.2%) of the placenta weighed less than 500 grams, twenty three (69.7%) weighed between 500-600 grams, while five (15.2%) weighed over 600 grams.

Seventeen (51.5%) of the babies were female and sixteen (48.5%) male. The mean birth weight was 3.29kg with a range of 2.50 – 4.00 kg.Thirty one (93.9%) of the birth weight were between 2.50 -3.90 kg while two (6.1%) were greater than 3.90 kg.

Table 2 shows the mean total nucleated cell count (TNC), CD34+ count, viability count of cells. The TNC count ranged from 2.90 - 21.10 x 10^6 cells/ml with a mean value of 11.14 x 10^6 ± 4.64 cells/ml. The mean value of CD34 + cells was3.89 x10^4 ± 1.48cells/ml with a range of 2.00 - 6.99 x10^4cells/ml. The mean viability of the cells was 90.00 ± 9.55% with a range of 60.0 - 98.20%.

Table3 shows the compared mean values of TNC count with maternal and neonatal factors.

TNC count decreases with increasing maternal age. However, the differences in mean were not statistically significant (p = 0.112). Similarly, TNC count tend to decline progressively with increasing maternal parity and they were statistically significant (p = 0.006).

Table 1A. Demographic parameters of maternal and neonatal factors

Neonatal and maternal factors	Frequency (n=33)	Percentage (%)
Maternal age (years)		
<25	1	3
25-35	25	75.8
>35	7	21.2
Parity		
1	5	15.2
2-5	11	33.3
>5	17	51.5
Birth weight (kg)		
2.5-3.9	31	93.9
>3.9	2	6.1
Gestational age (weeks)		
37 – 39	20	60.6
>39	13	39.4
Gender		
Female	17	51.5
Male	16	48.5
Mode of delivery		
CS	11	33.3
SVD	22	66.7
Placental weight (grams)		
<500	5	15.2
500-600	23	69.7
>600	5	15.1

CS - Caesarean Section, SVD - Spontaneous Vaginal Delivery

TNC count increases with increasing birth weight. However, the increament were not statistically significant (p = 0.598). Male babies had a higher mean value TNC count compared to females, but the difference in mean was not significant (11.4 $x10^6$ vs 10.9x 10^6; p = 0.774).Those that were delivered via SVD had a higher mean value of TNC compared to those delivered by ceasarian section (11.2 x 10^6vs 10.9x 10^6; p = 0.852). TNC tend to increase with increasing placental weight and gestational age; however, these were not statistically significant (p = 0.385, and 0.640 respectively).

Table 4 shows the mean value of CD34+ cells with maternal and neonatal factors. CD34+ count decreases with increasing maternal age, however, the differences were not statistically significant (p = 0.529). Similarly, CD34+ count tend to decline progressively with increasing maternal parity which was statistically significant (p = 0.006).

CD34+ count increases with increasing birth weight, however the increament were not statistically significant (p = 0.756). Male babies had a higher value mean CD34+ count compared to females but the difference was not significant (3.96 x 10^4vs 3.83 x 10^4, p = 0.794). Babies that were delivered via SVD had a higher mean value of CD34+cells compared to those delivered by ceasarian section (4.1 $x10^4$ vs 3.47 x 10^4; p = 0.250). CD34+cells tend to increase with increasing placental weight. However, these were not statistically significant (p = 0.307). CD34+ cells counts tend to decline with increasing gestational weight. More so, this was not statistically significant (p=0.783).

The bar Fig (1a-1g) below shows the mean values of TNC and $CD34^+$ cells with maternal and neonatal factors.

Table 1B. Mean and range distribution of maternal age, parity, gestational age, placental weight and birth weight

Maternal and neonatal factors	n	Mean ± SD	Range
Maternal age (year)	33	32.0 ± 4.40	23.0 - 40.0
Parity	33	3.18 ± 1.91	1–9
Gestational age (weeks)	33	38.84 ± 1.09	37–41
Placental weight(grams)	33	548.48 ± 71.20	400–650
Birth weight (kilogram)	33	3.29 ± 0.42	2.50 -4.00

Table 2. Mean and range distribution of CD34+cells and TNC

Variables	Mean ± SD	Range
TNC($x10^6$cells/ml)	11.14 ± 4.64	2.90 - 21.10
CD34+(x 10^4cells/ ml	3.89 ± 1.48	2.00 - 6.99

Table 3. Comparing the mean distribution of TNC (X10^6/ml) with maternal age, parity, gestational age, mode of delivery, gender, birth weight and placental weight

Neonatal and maternal factors	TNC x10^6/ml	F value	T value	P value
Maternal age (years)				
<25	13.40 ± 0.00	2.353		0.112
25-35	11.90 ± 4.32			
>35	7.93 ± 4.94			
Parity				
1	15.60 ± 2.05	6.161		0.006**
2-5	12.60 ± 5.40			
>5	8.90 ± 3.30			
Birth weight (kg)				
2.5-3.9	11.03 ± 4.70		-0.533	0.598
>3.9	12.85 ± 3.60			
Gestational age (weeks)				
37 – 39	10.80 ± 3.89		-0.472	0.640
>39	11.60 ±5.80			
Gender				
Female	10.91 ± 4.32		-0.290	0.774
Male	11.38 ± 5.09			
Mode of delivery				
CS	10.91 ± 4.81		-0.188	0.852
SVD	11.20 ± 4.66			
Placental weight (grams)				
<500	9.96±3.28	0.984		0.385
500-600	10.83±4.48			
>600	13.7±6.34			

***p-value<0.05, statistically significant*

3.1 Correlations of TNC and CD34+ cells with Maternal and Neonatal Parameters

Weak positive correlations were observed between birth weight, gender, mode of delivery, placental weight with TNC and they were not statistically significant. Moreover, a very weak negative correlation was found with gestational age (r = -0.0.20) and it was statistically not significant (p = 0.913). As shown in table 5, parity and maternal age had a negative correlation with TNC and that was statistically significant (r= -0.539; p = 0.001 and r =-0.358; p =0.041).

On the other hand, a weak positive correlation was observed between gender, birth weight, mode of delivery, placental weight and CD34+ cells and they were not statistically significant. Maternal age, gestational age and parity had a negative correlation with CD34+ cells, but only parity was statistically significant (r= -0.532; p = 0.001) as shown in Table 6.

4. DISCUSSION

Umbilical cord blood (UCB) is a novel and unique source of transplantable stem cells that can be used as therapy for diseases that require stem cell transplantation. CD34$^+$ cells, total nucleated cells, and viability count are the major parameters used in clinical practice to estimate stem cell dose infused during, haematopoietic stem cell transplantation (HSCT) and prediction of engraftment [23]. The dose of stem infused pre-HSCT is estimated by using flow cytometry for CD 34+ cells and automated full blood count analyzer for TNC [4,5,6]. This study demonstrated that UCB can be used as a source for stem cell for transplant in Nigeria since previous transplants were done with bone marrow stem cells.

The mean CD34$^+$ cells reported in our study is similar to the count seen among the Americans [26]. However a higher average CD34+cells count was reported among the Roman [27] even though these values are still within the reference interval reported in other studies [28,29].

The TNC obtained in our study is similar to those reported among the Indians [30] and the Spanish [28] populations but a bit higher among the Americans [26] and the Romans [27]. This showed the stability in terms of quantity in CD34+ cells and TNC components of umbilical

cord blood; hence UCB is a useful source of HSC transplant.

Some studies have shown that Caucasian infants had a higher number per ml of Colony Forming Unit (CFU), $CD34^+$ cells, TNC counts compared to Afro-American infants [30]. This probably explains the lower mean value observed in this study. More so, the findings in this study is also similar to those found in Spain and India [28,30]. Previous studies on total white cell count was found to be higher in Caucasian cord blood than African cord blood. This finding is also similar to TNC in Caucasian cord blood, although seemed to be higher in Africans. This probably could be due to geographical locations; high prevalence of acute and chronic malaria infection, medicinal herbs consumption and poverty with malnutrition in African countries [31]. Total nucleated cell has emerged as the most commonly reported parameter in addition to cord blood volume in estimating the potential of cord blood units for HSCT. This is seen in some studies which reported a strong association between TNC counts and engraftment, while some other authors suggested TNC as a single criterion for selecting cord blood for storage [27,32].

The number of TNC and CD34+ cells is used as dependent factor for selecting UCB units for transplantation. This study showed a positive correlation between TNC and CD34+ cells which is statistically significant ($p< 0.05$). Similar positive correlation was observed by Chandra et al. [33] and other studies [34,35]. The significance of positive correlation between CD34+ cells and TNC has enabled some centres to use TNC as a criteria in assessing the quality of the transfused stem cells especially in centres where assaying CD34+ cells are not available. More so, many of the cord blood banks rely on TNC counts as part of the quality check for cord blood banking [23,36].

Table 4. Comparing the mean distribution of CD34+ cells (X 10^4/ml) with maternal age, parity, gestational age, mode of delivery, gender, birth weight and placental weight

Neonatal and maternal factors	CD34+ cells x 10^4/ml	F value	t value	p values
Maternal age (years)				
<25	5.30 ± 0.00	0.650		0.529
25-35	3.94 ± 1.52			
>35	3.54 ± 1.48			
Parity				
1	5.24 ± 1.03	5.988		0.006**
2-5	4.37 ± 1.50			
>5	3.19 ± 1.48			
Birth weight (kg)				
2.5-3.9	3.87 ± 1.49		-0.314	0.756
>3.9	4.21 ± 1.82			
GA (weeks)				
37 – 39	3.95 ± 1.47		0.277	0.783
>39	3.80 ± 1.56			
Gender				
Female	3.50 ± 1.79		-0.263	0.794
Male	3.61 ± 2.27			
Mode of delivery				
CS	3.46 ± 1.15		-1.173	0.250
SVD	4.10 ± 1.60			
Placental weight (grams)				
<500	3.54 ± 1.46	1.229		0.307
500-600	3.76 ± 1.40			
>600	4.82 ± 1.80			

***p-values<0.05, statistically significant*

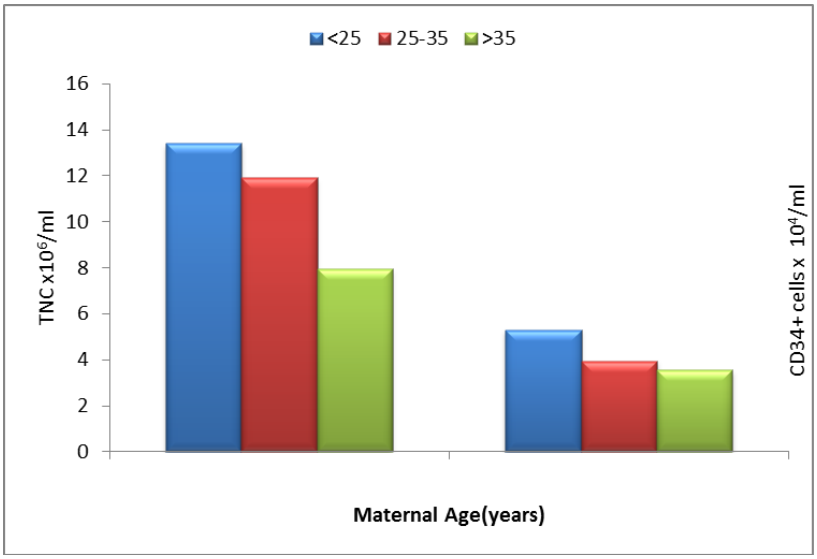

Fig. 1a. Showing the mean value of $CD34^+$ Cells and TNC with maternal age

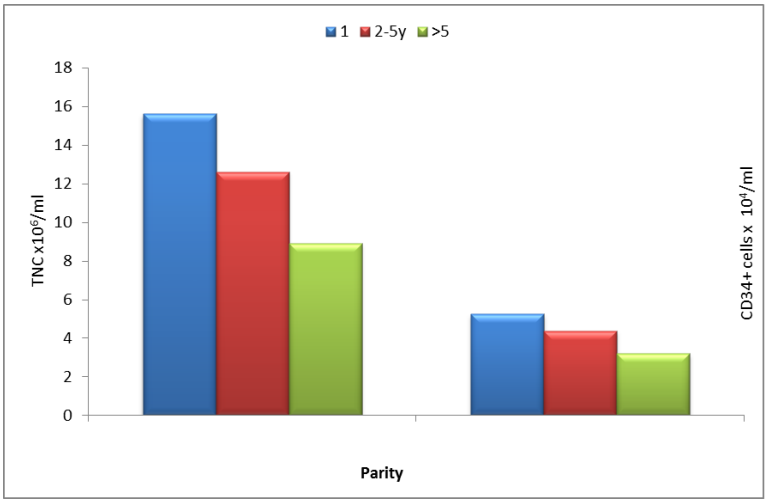

Fig. 1b. Showing the mean value of $CD34^+$ Cells and TNC with parity

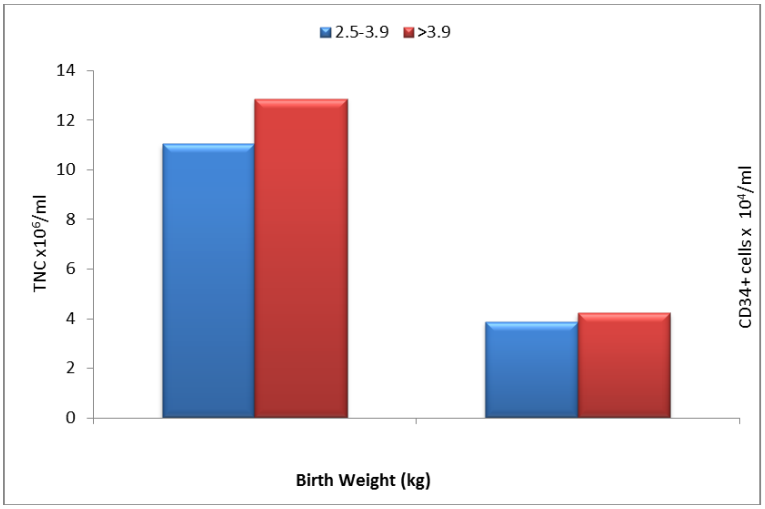

Fig. 1c. Showing the mean value of $CD34^+$ Cells and TNC with birth weight

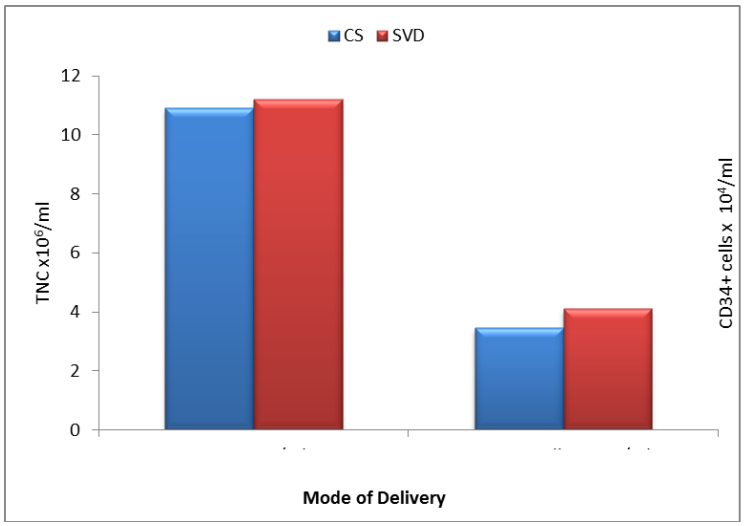

Fig. 1d. Showing the mean value of CD34$^+$ Cells and TNC with mode of delivery

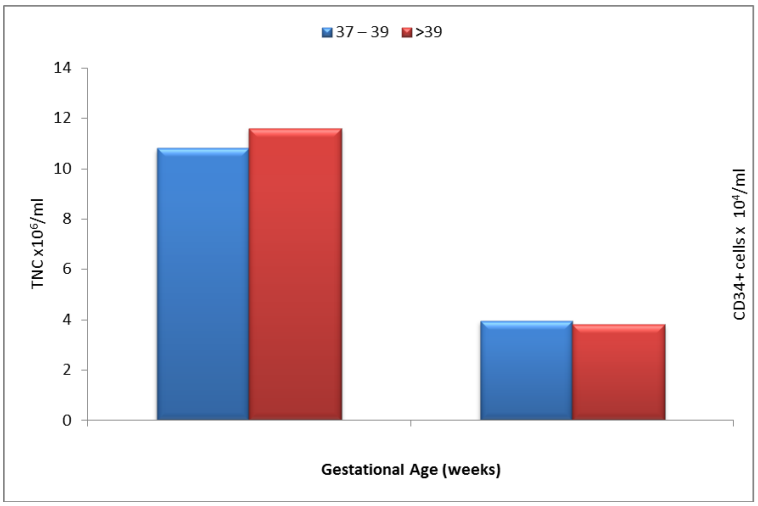

Fig. 1e. showing the mean value of CD34$^+$ Cells and TNC with Gestational Age (weeks)

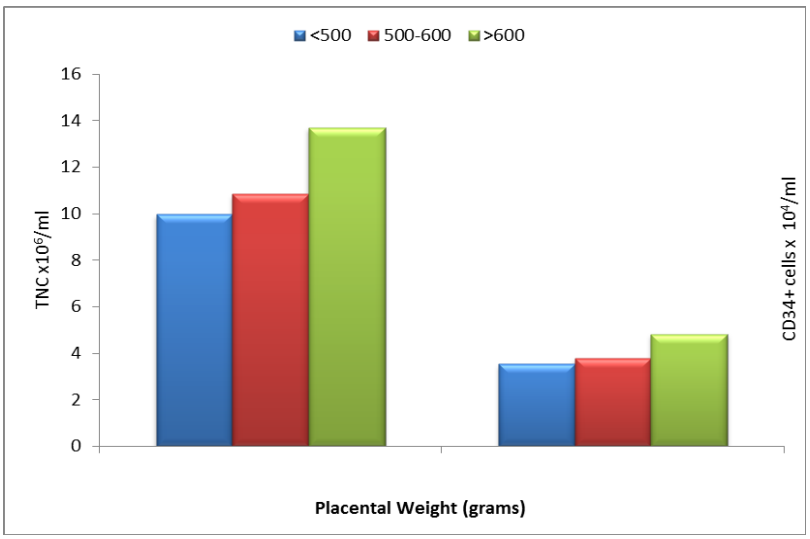

Fig. 1f. Showing the mean value of CD34$^+$ Cells and TNC with Placental weight (grams)

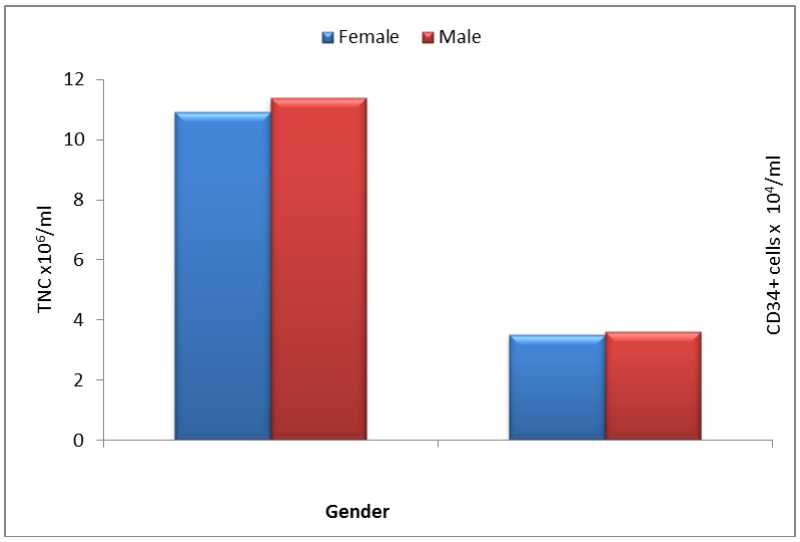

Fig. 1g. Showing the mean value of CD34$^+$ Cells and TNC with Gender

Table 5. Correlation of TNC with maternal age, parity, gestational age, mode of delivery, gender, birth weight and placental weight

Maternal and Neonatal Parameters	TNC	
	r	P
Maternal age	-0.3.58	0.041**
Parity	-0.539	0.001**
Gestational age	-0.020	0.913
Gender	0.052	0.774
Birth weight	0.095	0.598
Mode of delivery	0.034	0.852
Placental weight	0.226	0.205

Table 6. Correlation of CD34+ cells with maternal age, parity, gestational age, mode of delivery, gender, birth weight and placental weight

Maternal and Neonatal Parameters	CD34+ count	
	R	P
Maternal age	-0.177	0.325
Parity	-0.532	0.001**
Gestational age	0.049	0.785
Gender	0.047	0.794
Birth weight	0.056	0.756
Mode of delivery	0.206	0.250
Placental weight	0.241	0.176

Maternal and neonatal factors that can influence the higher yield of haematopoietic stem cells obtained from umbilical cord blood were also determined. The maternal factors are age and parity, while neonatal factors are birth weight, placental weight, gender, mode of delivery and gestational age.

The maternal age did not significantly affect the TNC and CD34+ cells count of cord blood. This observation was similar to that reported by Ballen et al. and Joseph and collaborators [29,37]. A previous report showed that women older than 25 years of age carry more TNCs, and lower maternal age was associated with a higher CD34+ cell concentration [30] However, in this study the younger mothers who were less than 25 years had higher values of TNC and CD34+ cells than the older age groups. This probably might be due to the observed inverse relationship between CD34+ cell count and maternal age [23].

Maternal parity has been reported to impact on TNC and CD34+ cells yield in cord blood. In this study, there was a steady decline in TNC and CD34+ cells yield with increasing maternal age. Cord blood yield from primiparous mothers was signifcantly higher. Similarly, a negative correlation was observed between parity with TNC and CD34+ cells, athough only the later was statistically significant. Ballen et al. [38] showed that female babies born of primiparous mothers have a higher TNC and CD34+ cells which may probably be due to prolonged labour associated with first babies [39], this may have been reflected in this study. On the other hand, Joseph et al and Nakagawa et al. [40,41] did not report such association.

Gestational age shows no significant correlation with TNC and CD34+cells, however babies born within 37-39 weeks had a higher TNC and CD 34+ cells. This was observed in similar studies [18,42]. These relationships are probably due to

the mobilizing signals produced by placenta tissue during foetal development.

Birth weight shows no significant correlation with TNC and CD34+cells, however babies with birth weight greater than 3.9 grams had a higher TNC and CD34+ cells. This has been observed by other researchers in similar studies [37,42], On the contrary, some other studies showed a significant positive correlation between birth weight and TNC and CD34+cells [29,43] While Choong et al. [44] reported an association between birth weight with TNC only. In the index study most of the babies whose cord blood were studied had normal weight. If babies with low birth weight and macrosomia were included, the impact of the birth weight on cord blood TNC and CD34+ yield would be better appreciated. This significant observation may be largely due to the birth weight, which could be directly affected by placental weight [45] Thame et al. [45] suggested that placental weight may be a more reliable predictor of size at birth than foetal measurements, and may be useful in the early identification of a foetus at risk in the perinatal period. However this study showed that placental weight has effect on TNC only and no effect on viability count and CD34+ cells, however placental with much higher weight (>600 g) have higher values of TNC and CD34+ cells, while those with weight (<500 g) have lower values of cell count. A larger sample size in this study may have reflected the significance of placental weight.

Neonatal sex has also been reported to have an effect on the cord blood yield of TNC and CD34+count. This study found a slightly higher TNC and CD34+ count in male babies even though it was not statistically significant. Jan *et al.*[46] in their study have reported that cord blood collected from male babies had higher CD34+ cells and blood volume but low TNC counts. A study by Aroviita et al. [34] found significantly more CD34+ cells in male babies. Some other studies showed that female sex was associated with a higher TNC count [29,44].

There was no statistically significant relationship between cell count (TNC and CD34+cells) and mode of delivery though babies delivered even through spontaneous vaginal delivery (SVD) had a higher TNC andCD34+ cells than those delivered by Caesarean section. A similar work by Aroviita et al. also showed no correlation between cell count and mode of delivery [34]. In accordance with Sparrow, he observed that TNC was higher in cord blood collected from babies delivered by caesarean section [46]. This difference in the reports of the mode of delivery could be due to a foetal response to the stress of labour [47].

5. CONCLUSION

This study showed that CD34+ cells and TNC of UCB at the University of Benin Teaching Hospital are within the acceptable range for Haematopoietic Stem cell transplantation. Maternal parity was found to have an inverse relationship with TNC and CD34+cells yield from neonatal cord blood. A positive relationship between TNC and CD34+ cells reflects the possibility of using TNC as an alternative to CD34+ estimation in centres without facilities for estimating CD34+ cells. However, neonatal parameters seem not to significantly impact much on TNC and CD34+ cells yield.

6. RECOMMENDATION

We suggest the use of total nucleated cell count values in place of CD34+cell count in assessing the quality of umbilical cord blood for haematopoietic stem cell transplant for the management of diseases amenable with this procedure.

CONSENT

Informed and Witten consent for UCB collection was obtained from healthy mothers with uncomplicated pregnancies receiving care at the Department of Obstetrics.

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.

ACKNOWLEDGEMENT

We appreciate Mr Moibi O. (laboratory scientist) and Dr Mabayoje O, haematologist from LAUTECH for their contribution in haematologic analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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