FACTOR PREDICTING TOTAL NUCLEATED CELL COUNT IN CORD BLOOD UNITS COLLECTED AT KING ABDULLAH INTERNATIONAL MEDICAL RESEARCH CENTER CORD BLOOD BANK

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Abstract - Stem cells originating from neonatal cord blood have been used worldwide in transplant medicine to treat various diseases. Stem cells efficacy in the umbilical cord blood (UCB) can be predicted by the number of total nucleated cells (TNC). To optimize the clinical use of stem cell in our population, this study addresses several variables affecting TNC count.

This is an observational cross- sectional study conducted at King Abdulaziz Medical City. From 2012 to 2014, 957 UCB units were collected from consented mothers by trained personnel using standard procedure. Data analysis of clinically accepted CBUs was correlated with maternal and infant factors. The demographic data of our subjects included maternal age(means ± SE: 29±0.18) and gestational age(39±0.04). The [Median (IQR)] of the mother's gravidity [3(4)], parity [2(3)], abortion [0(1)] and the number of living children [2(3)]. Based on the TNC accepted level of banking which is at least 90x107 cells, 188 CBUs (19.64 %) were rejected from a total of 957 units. Of the 17 maternal and infant variables evaluated, three factors demonstrated a statistically significant predictive value of accepted TNC level. Cord blood volume was the best predictive factor (p-value=<0.0001). Consequently, newborn birth weight (p-value =0.025) and method of delivery (P-value= 0.002).

Several maternal, neonatal and obstetric factors appear to play a major role in predicating accepted TNC count level which can be used to improve the criteria of the donation of stem cells in cord blood unit.

I. INTRODUCTION

Hematopoietic stem cells are multipotent cells that can differentiate to all types of blood cells. Hematopoietic stem cells have been used clinically to treat many hematological diseases including leukemia, lymphoma and anemia and that's by transplantation, a procedure that take the stem cells and insert it into a patient for therapeutically purpose. There are two main sources of hematopoietic stem cells: adult stem cells (bone marrow, peripheral blood vessels) and umbilical cord blood. Adult stem cells are extracted mainly from the pelvic bone marrow where stem cells is concentrated in the adult, also from the blood vessels which is enhanced by the injecting G-CSF into the patient to mobilize these stem cells from the bone marrow to the peripheral blood and then collect them for transplantation .Incidence of graft versus Host Diseases (GvHD) is the main obstacle to get a successful transplantation of bone marrow and peripheral blood vessels stem cells.

Umbilical cord blood (UCB) is an important source of stem cells and an alternative therapy to bone marrow transplantation in the absence of an HLA-matched bone marrow donor. Cord blood (CB) have been introduced in the medical field since the successful transplant of CB for a fanconi anemic patient in 1988. CB is the remaining blood in the umbilical cord and placenta after delivery. It is collected from the vein of the umbilical cord. There are two methods for CB collection, one before the delivery of the placenta (in-utero), the other after the delivery of the placenta (ex-utero). After CB collection, processing and screening, CB are cryopreserved for future usage in a cord blood bank. Unfortunately, usage of cord blood as a therapy is limited for children and young adults due to the small volume of blood in the umbilical cord. The stem cells efficacy in the UCB can be predicted by the number of total nucleated cells (TNC) and CD34'. Since the CB's volume is very small, both TNC and CD34 are very critical for the quality of the unit, as the number of TNC should be > 3.7X107/kg to be suitable for transplantation. Many factors can affect the quality of the CBUs including both Mothers' and neonates' factors.

The aim of this study was to investigate different variables that can affect the numbers of TNC in the collected CBUs to improve the criteria of CB donation.

II. METHOD

This is a cross-sectional study with a convenient and consecutive enrolling technique. It included 957 mothers who delivered at National Guard Hospital Affairs (NGHA) from 2012 to 2014 and were willing to donate their cord blood. We excluded mothers under 18years, mothers with cancer, inherited, or transmittable diseases, mothers infected with malaria within the last three years, pregnant with multiples, If one of the parents had received an organ, tissue transplant or blood transfusion or got a tattoo within the last 12 months. We also excluded the donation if the weight of the baby was < 2500g (2.5kg), the baby was born premature <34 weeks, any sign of infection or birth defect, Apgar score for the baby less than 7-8/10 in 5 minutes, the umbilical cord showed any abnormalities and the collected blood volume was <50 ml.

The birth weight of the newborn was measured by trained nurses using a clibrated digital scale (SECA). The placenta weight was measured by trained midwives according to standard procedures issued by the National Board of Health. Each placenta was weighed with a regularly calibrated digital scale (AE. ADAM) between 15 to 20 minutes after delivery, including membranes and umbilical cord.

All mothers were interviewed, consented and registered by the cord blood bank coordinator. (Signing informed consent is a process by which parents voluntarily conforms their willing to donate cord blood of the infant upon delivery , after having been informed of all relevant information required for taking decision to participate in the cord blood donation program). Once mothers were consented , the medical history was reviewed to complete the acceptability assessment checklist of the donor to decide whether to include or exclude the mother from the study. After a successful collection, the blood was transferred to the lab for processing.

Cord blood collection:

Cord blood is collected by the cord blood bank staff nurses or midwives. The collection is done by a collecting kit (closed system) containing anticoagulants. The blood is collected from the vein of the umbilical cord through ex-utero (after the delivery of the placenta) or in-utero (before the delivery of the placenta) collection from vaginal or caesarean deliveries. The collected unit will then be sent at room temperature to the cord blood bank laboratory for processing.

Cord blood processing:

At the laboratory the cord blood units will be processed using a closed system to get red of the plasma and red blood cells. However before that the unit will be checked for criteria which include:

i. Physical check of the bag integrity, sample ID verification with attached documents, absence of clumps and clots;
ii. Cord blood unit's volume
iii. The number of total nucleated cells using a hematology analyzer (AcT diff, Beckman Coulter, Fullerton, CA, USA).

All final CB units are tested for transmissible diseases HBV, HCV, HIV, HTLV I-II, NAT, syphilis, and haemoglobinpathy. In addition CB units are tested for sterility by microbiological testing (Bactic Alert), complete blood count (CBC) including differential count (AcT diff, Beckman Coulter, Fullerton, CA, USA), CD34+ stem cell number and viability are counted using labeled antibodies (flow cytometry, BD BioScience, San Jose, CA, USA).

Statical analysis:

Sample size was collected based on 95% confidence level. Data was entered into SPSS software, simple statistics of demographics variables(means, standard deviation, frequency, percentage) collected on mothers and infants were calculated and analyzed. Multiple logistic regression was carried out to estimate the factors predicting stem cell count in cord blood units.

III. RESULTS

the median(iqr) of the mother's gravidity was 3(4), parity 2(3) , abortion 0(1) and the number of living children 2(3). obstetrics variables are summarized in table 2 .the means ± se of the continuous variables included maternal age 29±0.18, gestational age 39±0.04 , cord blood volume 74.22 ±0.84, newborn weight 3237.13 ± 12.24, placenta weight 570.79 ± 3.70, maternal wbc 10.09 ± 0.12, maternal rbc 4.30 ± 0.01, hemoglobin 119.17 ± 0.4, cord blood wbc 8.49 ±0.11, total count of nucleated cells 939.77 ± 17.05. the factors in this study were divided into two categories: infant and maternal factors . the mean± se of the maternal and fetal categories are summarized in table 3.

Out of the 17 infant and maternal variables evaluated, three factors demonstrated a statistically significant predictive value of accepted TNC level. Cord blood volume was the best predictive factor (p-value=<0.0001). Consequently, newborn birth weight (p-value =0.025) and method of delivery (P-value= 0.002).

Proportional odds model was used to identify the significant predictors of the total nucleated cell count (TNC) acceptance levels. The model included only covariates that showed significance at the bivariate level and significance was declared at $\alpha < 0.05$. The predictive analysis is summarized in Table 4

IV. DISCUSSION

The first cord blood bank was established in 1992. Now a day many cord blood bank centers locally and internationally have been built to store the CB for future therapy. Random collection of cord blood without following specific criteria to select high quality units, are time and cost wasting. To minimize that, many researches have investigated the factors that can affect the quality of cord blood.

Some studies have addressed only few variables and others have set some chronic diseases as variables, whereas our research investigated 17 variabled from healthy mothers and infants.

In an attempt to improve the criteria of the donation of stem cells in cord blood unit, we addressed the following variables: mother's age, gestational age, gravidity, parity, number of abortions, number of living children, placenta weight , newborn weight , blood volume , method of delivery, white blood cells count, pre red blood cells count, hematocrit, and studied their association with TNC volume.

Results of this study, shows that cord blood volume is the best predictive value of TNC count(p-value=<0.0001), similar to Carolinas Cord Blood Bank (CCBB) results and other studies. Furthermore, for every 10 grams deviation from the mean cord blood weight, there was a 2.6% chance more likely to produce TNC counts to be accepted for clinical use compared to research use or rejected levels.

A statistically significant correlation exist between clinically accepted TNC count and method of delivery (p-value =0.025). We a showed that delivering mothers who underwent C-section are 40% less likely to produce TNC counts to be accepted for clinical use compared to research use or rejected levels. While this claim was also supported from previous data (1,2) , mode of delivery did not show any significant influences in St John Hospital Guild Cord Blood Collection Program and CCBB.

There was no significant influence in other variables like gestational age, parity, infant sex in comparison to the results of the St John Hospital Guild Cord Blood Collection Program. Also on the contrary to the CCBB, the maternal age showed no effect in the TNCs.

Similar to data from previous studied , the rest of the variables that have been studied like gravidity , abortion, living children, placenta weight, white blood cells count, red blood cells count and hematocrit , all had no effect on TNC.

V. TABLES

Table 1: Clinical Characteristics of the Study Subjects

Variables		Statistics (N(%))
Gender	Male	530 (55.32)
	Female	428 (44.68)
Method of Delivery	Vaginal	831 (86.74)
	C-section	127 (13.26)
TNC_Acceptance Levels	Accepted For Clinical Use	532 (55.59)
	Accepted For Research Use	237 (24.76)
	Rejected	188 (19.64)

Table 2: Clinical Characteristics of the Study Subjects

Variables	Variables Statistics[Median(IQR)]
Gravida	3 (4)
Parity	2 (3)
Number of Abortion	0(1)
Number of Living Children	2(3)

Table 3: Clinical Characteristics of the Study Subjects

Variables	Variables Statistics [mean ± SE]
Gestational Age	39 ± 0.04
Mother's Age	29 ± 0.18
Cord Blood Volume	74.22 ±0.84
Newborn Weight	3237.13 ± 12.24
Placenta Weight	570.79 ± 3.70
Maternal WBC	10.09 ± 0.12
Maternal RBC	4.30 ± 0.01
Hemoglobin	119.17 ± 0.44
Cord Blood (WBC)	8.49 ±0.11
Total Count of Nucleated Cells	939.77 ± 17.05

Table 4: Predictive Analysis.

Variables	OR	95% CI		P-value
		Lower Bound	Upper Bound	
Newborn Gender (Male Vs. Female)	0.92	0.69	1.24	0.622
Method of Delivery (C-Section Vs. Vaginal)	0.61	0.40	0.94	0.025
Newborn Weight	1.40	1.14	1.72	0.002
Cord Blood Volume	2.60	2.32	2.92	<0.0001

Note: Probabilities modeled are cumulated over the lower ordered value (Accepted for clinical use)

CONCLUSION

With the expansion of umbilical cord blood banking, we recommend to set a criteria for the donation of stem cells from UCB and suggest other studies to look for other variables from different aspect of the mother and the infant like nutrition, race and psychosocial status. Currently, the study was limited in the central region in Riyadh specifically in (NGHA), we encourage to study the factor predicting TNC among different region.

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