PLACENTA UMBILICAL CORD BLOOD: DETERMINATION OF THE SUITABILITY FOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION AT THE UNIVERSITY OF BENIN TEACHING HOSPITAL NIGERIA

BY

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A DISSERTATION SUBMITTED TO THE NATIONAL POSTGRADUATE MEDICAL COLLEGE OF NIGERIA IN PART FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE FELLOWSHIP OF THE COLLEGE IN THE FACULTY OF PATHOLOGY.

NOVEMBER, 2017.
DECLARATION

I hereby declare that this work is original. This work has not been presented to any other college for a Fellowship, nor has it been submitted for publication.

........................................

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DEDICATION

This work is dedicated to my creator and my Lord Jesus and to my family for being there always
ACKNOWLEDGEMENT

I thank my family for their support and encouragement which has been my source of inspiration.

Special thanks go to my supervisors, Prof G.N Bazuaye and Dr. E. Enabudoso without their meaningful contribution, this study would not have been possible.

I thank my teachers, Prof N.K.D Halim, Prof M.E Enosolease, Prof O.A Awodu, Prof C.E Omoti, Dr. J. Obieche and Dr. B. Nwogoh, for their advice and guidance.

Special thanks to Dr. B. Nwogoh, Dr. S. O. Oguntuase and Dr. Carl Umakhihe, for their assistance and technical support

I am grateful to my colleagues Drs. Iheanacho, Ezewenyi, Nnachi, Adewonyin, Ezire, Adeyemi, Oguntuase, Idubor, Iyawe, Udubor, Dirisu, Onoh, Ukwade, Owolabi and Okuonghase for their contribution and support during the course of this study.

I thank Dr. V.O Mabayoje and Mr. Muhibi from LAUTECH for all the technical support they gave to me.

I thank the staff of the haematology laboratory, Obstetrics and Gynecology department for their co-operation and the assistance I got during this study.

I specially appreciate my husband Oladotun, my children Oluwasemilore, Omoloorun, Adewumi, my grandmother Patricia Aigbe, my parents Barr. and Mrs. Osarumwense, and my siblings for their love and prayers for me.
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ABSTRACT

Background: 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 and adolescents. It has several advantages as it is easily available, involves non-invasive collection, better tolerance across the HLA barrier and less complicated by GVHD. Following the first Umbilical cord blood transplantation (UCBT) in 1988, over 1000 umbilical cord blood transplantation (UCBT) have been done. CD34+cells, total nucleated cells (TNC) and viability count are the main parameters used in clinical practice to estimate stem cell dose infused during transplant and also used to predict engraftment.

Objective: The present study was carried out to assess the suitability of umbilical cord blood stem cells for Haematopoietic stem cell transplantation by using CD34+ cells count, total nucleated cells count and viability of the cord blood stem cells

Materials and methods: A cross sectional study conducted in University of Benin Teaching Hospital (UBTH), Benin City. A total of thirty-three umbilical cord blood were collected from the placenta after delivery. CD34+ cells were enumerated using flow cytometer. Viability count was done with molecular exclusion dye 7-aminoactinomycin D using flow cytometer and Haematology Analyser was used to assess TNC.

Results: CD 34+ cells count ranged between 2.0 - 6.99 x 10⁴cells/ml with a mean value of 3.89 ± 1.48 x 10⁴cells/ml. Mean value of TNC was 11.14 ± 4.47 x 10⁶ cells/ml with a range of 4.80-21.10 x10⁶ cells/ml while the mean value for viability count was 90.0 ± 9.57 % with a range of 60.0 - 98.2 %. The study showed a positive correlation between CD34+ cells and TNC count (r = 0.760, p=0.000) and a negative correlation between viability and processing time (r = -0.859,
p=0.000). In addition, maternal parity showed a significant inverse relationship with TNC and CD34+ cells.

**Conclusion:** The study showed that CD34+ cells, TNC and viability count of UCB are within the acceptable values for transplantation using cord blood. There is a direct positive correlation between TNC and CD34+ counts. In Nigeria, the use UCB technique in HSCT can be explored as it promises immense benefits in stem cell transplantation as obtainable in more organized centres.
CHAPTER ONE
INTRODUCTION

Haematopoietic stem cells (HSC) are defined as cells capable of differentiating into several progenitor cells of different cell lines and as having the capacity for extensive self-renewal or self-maintenance. The progenitor cells are members of a cell population with little or no self-renewal capacity but 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 so that they can assume biological functions in the recipient’s body.² Malignant and non-malignant disorders such as acute and chronic leukaemia, lymphomas, solid tumours, immune deficiencies, inborn errors of metabolism and genetic diseases can be cured with HSCT.³ Stem cells can be harvested from patient (autologous) or from related or unrelated (allogeneic) donors.⁴ Some 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.⁴,⁵ Unfortunately, only 30% of patient’s have an HLA identical sibling donor and identification of matching unrelated donors, particularly for minorities, can present an exceptional challenge. The transplantation of umbilical cord blood (UCB) represents the most recent strategy to expand the potential donor pool while maintaining an acceptable level of treatment related complications.⁶

Knudtzon initially studied the presence of primitive and committed haematopoietic progenitor cells in the cord blood, than followed by Ogawa and other investigators.⁷,⁸, ⁹, ¹⁰ Broxmeyer et al established that umbilical cord blood contains sufficient number of
haematopoietic stem cell and progenitor cells that can be used for autologous and allogeneic haematopoietic reconstitution. 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.

The number of functional haematopoietic stem cells in an UCB unit is ten times less than in a bone marrow graft but their proliferative capacity is superior to that of cells in an adult BM or peripheral blood. Following myeloblastic therapy UCB stem cells are also capable of providing both haematological and immunological reconstruction. UCB with its apparent lesser immunogenicity was evidenced by the lower incidence of severe Graft versus Host disease (GvHD) reported after related and unrelated UCBT that was expected even in mismatched transplants. Despite the wider HLA disparity between grafts and recipients, the relatively easy collection procedure of CB units has permitted an enormous expansion of the donor pool and representation of ethnic minorities. UCB offers additional advantages over BMT in unrelated transplantation such as a low transmission rate of infectious and genetic disease, the immediate availability of the product avoiding the risks for the donor and loss of registries and a higher probability and considerably faster identification of suitable units.

The greatest limiting factor in the use of umbilical cord blood for transplantations is the number of stem cells available in any given collected unit, thus its limited dose compromises the outcome of adult UCB transplantation. The number of nucleated cells infused per kilogram of recipient body weight correlated with neutrophils recovery time. The lowest safe dose of nucleated cells to provide durable engraftment is 2 x 10^7 nucleated cells per kilogram body weight (NC/Kg). The engraftment, treatment related mortality and survival of recipients of UCBT are associated with the number of total nucleated cells and the dose of CD 34 positive (+) cells. It has been shown that 1.7 x 10^5 CD34+ stem cells /kg body weight
and nucleated cells >2 x10^7/per kilogram body weight with viability of the nucleated cells greater than 75% are needed for UCB transplantation. Therefore, the number of CD34+ cell and the total nucleated cells are the determining factor for the suitability of cord blood for HSCT. 17, 18, 19, 20

In Nigeria, though umbilical cord blood as a source of stem cell transplantation is still far-fetched, 21 there is hope that in the nearest future the possibility of Placenta umbilical cord transplantation could occur in Nigeria, following the success of Bone Marrow Transplantation for two sickle cell anaemia patients. 22

RELEVANCE OF THE STUDY

Umbilical cord blood is a critically useful source of haematopoietic stem and progenitor cells for treatment of a wide variety of malignant and non-malignant disorders. The practice of UCB donation and banking is on the increase in many parts of the world. 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. 23 An average of about 100ml of blood can be harvested from a placenta, and in Nigeria, about 5 million children are born annually. This amounts to about 500,000,000 ml of blood wasted on annual basis because no harvesting is done. If UCB is put to use in Nigeria, it could become an important source of stem cell for HSCT. 24, 25

Cord blood provides a readily available graft for the recipient that does not have suitable matched related or unrelated donors. 23 Sickle cell anaemia is an example of a genetic disease which can be treated with HSCT. Nigeria has the largest burden of sickle cell anaemia (SCA) in Africa; at least 40 million Nigerians are heterozygous (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.\textsuperscript{22,26} At present, the only viable option for cure of sickle cell disease (SCD) is HSCT.\textsuperscript{22,27} The two successful allogeneic HSCT done in Nigeria were from HLA matched sibling donors in 2011 and 2013 respectively.\textsuperscript{22}

Previous studies on haematological parameters, showed that Caucasian values of total white blood cells count are higher than that of the black race,\textsuperscript{28} hence it will be of great relevance to determine if the TNC of Nigerian babies umbilical cord blood will also differs from those of Caucasian origin.

There is paucity of published researches carried out locally on suitability of placental Umbilical cord blood for stem cell transplantation. This work will seek to add to local database on the parameters of haematopoietic potential from UCB, such as viability, total nucleated cell counts and CD34+ cell counts.
AIM AND OBJECTIVES

Aim
To evaluate the suitability of placenta umbilical cord blood for stem cell transplantation at the University of Benin Teaching Hospital in order to ascertain its relevance as a source of stem cell in Haematopoietic stem cell transplantation therapy for malignant and non-malignant diseases.

Objectives
1. To determine the yield of CD34+ cells in umbilical cord blood
2. To determine the viability of stem cells in umbilical cord blood.
3. To determine the association between total nucleated cells and CD34+ cells in cord blood.
CHAPTER TWO
LITERATURE REVIEW

Overview of Haemopoietic Stem Cells

Haematopoiesis is a complex and highly orchestrated process by which pluripotent HSCs differentiate into functional blood cells. In this hierarchical proliferation and differentiation process, self-renewing HSCs first differentiate into multipotent progenitor cells (MPPs). The MPPs further differentiate into lineage-committed lymphoid cell or erythrocytes.\textsuperscript{1,29}

Morphologically, haematopoietic stem cells are medium-sized mononuclear cells with a high nuclear-cytoplasmic ratio, basophilic cytoplasm with no granules, and prominent nucleoli: (flow cytometry is used for identification and counting).\textsuperscript{30}

HSC and progenitor cells express unique surface markers that make them distinguishable from other cell types. The markers are CD34\textsuperscript{+}, CD38\textsuperscript{-}, CD90\textsuperscript{+}, CD45RA\textsuperscript{-}, CD49\textsuperscript{+}. All HSC and progenitor cells have CD34 cells expressed as a surface marker. It plays a central role in HSC and progenitor cell recognition.\textsuperscript{30}

CD34\textsuperscript{+} is a highly O-glycosylated transmembrane protein with a molecular weight of 115 kDa. The gene is located at band1q32. CD34\textsuperscript{+} cells comprise approximately 1\% of bone marrow mononuclear cells and UCB and it is the surrogate marker of the haematopoietic progenitor and stem cell content of cord blood transplants. CD90 is another important cell surface marker expressed on early stage haematopoietic stem cells.\textsuperscript{30,31}

HSCs are maintained as a quiescent population of cells and their numbers in the BM and circulation are highly regulated. The mechanism controlling their homing to the bone marrow, self-renewal and differentiation are thought to be influenced by a diverse set of cytokines, chemokines, receptors and intracellular signaling molecules.\textsuperscript{32,33,34}
Haematopoietic Stem Cell Transplantation

HSCT is the transfer/transplantation of haematopoietic stem cells from a donor to a recipient so that they can assume biological functions in the recipient’s body and then carry out these functions permanently.\(^2\)\(^,\)\(^30\) It is the infusion of HSCs with the intention to replace the pre-transplant recipient haematopoietic system.\(^35\) HSCT is an established therapy today and has experienced rapid expansion and improvement over the last decade. It is a curative therapy for many severe acquired and congenital disorders of the haematopoietic system and for chemo-sensitive, radiosensitive or immunosensitive malignancies.

HSCT is associated with significant morbidity and mortality and represents one example of high-cost and highly specialized medicine. The benefits of HSCT are;

1. Restoring bone marrow function in a patient with congenital deficiencies or acquired malfunction.
2. It shortens the period of severe pancytopenia in the context of high dose chemotherapy/radiotherapy in autologous transplant.
3. Allogeneic HSCT provides a powerful targeted anti-tumour effect in the context of a graft-versus-host or graft-versus-tumour reaction for the treatment of haematological or non-haematological malignancies
4. HSCT can reset ontogenesis of the immune system in the treatment of autoimmune disease
5. HSCT can induce tolerance to donor tissue in combination with solid organ transplantation.\(^35\)
Types of HSCT

A. Autologous:
Autologous transplantation is a process of infusing patient's own stem cells, which was harvested earlier as a rescue therapy after high-dose myeloablative therapy. It offers the advantage of not causing graft versus host disease [GVHD], does not require immunosuppressant, less infection, especially post engraftment period and no tissue incompatibility between the donor and the host. The disadvantages of an autologous graft are no graft-versus-tumour effect, hence higher risk of disease relapse/progression than allogeneic HSCT, impaired ability to collect HSC if patient is heavily treated with prior chemotherapy and graft may be contaminated with tumour cells.36, 37, 38

B. Allogeneic HSCT;
Allogeneic transplantation is a process of infusing the donor’s HSC to a recipient of the same specie but not genetically identical (with the rare exceptions of transplantation between identical twins known as syngeneic transplantation). Non-identical individuals differ in their human leukocyte antigens (HLAs). The HLA genes are inherited haplotypes, located in the short arm of chromosome 6 and contain a cluster of genes known as Major Histocompatibility Complex (MHC). Their role is to bind intracellular peptides and ‘present’ them to T lymphocytes for antigen recognition. The immune system uses these HLAs to distinguish between “self” and “non-self”. There are two types of HLA proteins: class1 and class 11. Class 1[HLA-A, -B, and –C] present antigen to CD8+ T cells and class 11 molecules (HLA-DR, -DQ, and –DP) present to CD4+ T cells. Allogeneic grafts must match most, if not all of the six to ten major HLA antigens between host and donor. In HLA identical sibling donors, typing at HLA–A, B and DR is sufficient to determine HLA
compatibility between donor and recipient. While for unrelated donors, further typing for HLA –C, DQ and DP is required for compatibility between donor and recipient. The advantages of allogenic HSCT are the potential for a graft versus tumour response, which can be an important contribution of achieving and maintaining complete remission, no risk of graft contamination by tumour cells. The disadvantages include the possibility of developing potentially lethal graft versus host disease (GVHD) and they are limited by the availability of immunologically-matched donors.37-42

Allogeneic HSCT is currently the only therapeutic approach for several lethal malignant and non-malignant disorders. In an attempt to reduce transplant related toxicity, placenta umbilical cord blood has emerged as an alternative source of stem cells for patients in need of allogeneic transplantation.13

**Haploidentical HSCT;**

This is a type of allogenic haematopoietic stem cell transplantation. In haploidentical SCT, the donor is with only a single HLA haplotype match, usually a sibling, parent, or offspring. The advantages are; most patients have a readily available donors and the strong graft-versus-tumour effect may be possible with HLA disparity.40 However, the widespread acceptance of haploidentical HCT remains hampered by the prolonged immune reconstitution and a high risk of serious infection.43,44

**SOURCES OF STEM CELLS FOR HSCT**

Three major sources of stem cells have been approved for haematopoietic reconstitution; - the bone marrow, peripheral blood and cord blood. However, other sources like adipose tissue are still experimental.30, 43, 45
THE BONE MARROW

Bone marrow is the primary site of haematopoietic stem cell in adults.\textsuperscript{30,46} It is the traditional source of HSCs for allogeneic and autologous transplantation. The marrow is aspirated by repeated placement of large bore needles into the posterior iliac crest, generally 50 to 100ml aspirations simultaneously on both sides, while under regional or general anaesthesisia. The lowest cell dose to ensure stable long-term engraftment has not been defined and a typical collection standard contains more than $2 \times 10^8$ nucleated marrow cells/kg recipient body weight, while volume of 20 mL/kg donor body weight is considered safe.\textsuperscript{44}

PERIPHERAL BLOOD PROGENITOR CELLS

Haematopoietic stem cells are present in the blood at very low levels; however a number of different stimuli including chemotherapy, various haematopoietic growth factors and inhibitors of certain chemokine receptors (G-CSF, granulocyte-monocyte colony-stimulating factor (GM-CSF), interleukin (IL)-3, thrombopoietin and the CXCR4 antagonist AMD3100) result in the mobilization of HSCs from marrow to blood and the HSC are collected by apheresis.\textsuperscript{44}

UMBILICAL CORD BLOOD

In the late 1980s UCB was recognized as an important clinical source of HSC.\textsuperscript{47,48} Foetal blood immediately prior to delivery has been shown to contain haematopoietic progenitor cells at similar or higher levels than those in adult BM.\textsuperscript{11,30,49} The number of nucleated cells and CD34+cells per kilogram body weight of the recipient is approximately one log lower compared with bone marrow as stem cell source. Several studies have shown the importance of a high CB cell dose, more than $2 \times 10^7$ nucleated cells/kg, CD34+ more than $1.7 \times 10^5$/kg and viability count $> 70\%$ are recommended.\textsuperscript{17,30,40}
UCB is usually discarded but it is a rich source of stem /progenitor cells. It can be easily collected without any danger or inconvenience to the donor (mother and baby).30,50,51,52

**Methods of Collecting Cord Blood:** - There are two main methods for collecting cord blood from the umbilical vein: before the delivery of the placenta (*in utero*) and after the delivery of the placenta (*ex utero*). *In utero* cord blood collection is performed after the infant has been delivered but before delivery of the placenta. A closed collection system is used to reduce the risk of bacterial and maternal fluid contamination. In *ex utero* cord blood collection, it is performed after delivery of the placenta. The placenta is suspended on a stand or frame and blood is collected by gravity from the venipuncture site.4

**Reduction of UCB Bulk: Preparation of Leukocyte Concentrates (LCs)**: - Hydroxyethyl starch (HES) in a concentration of 6% is added to the anticoagulated UCB in a ratio of 1:5 to enhance its low red blood cell sedimentation rate. A leukocyte rich supernatant is then separated by centrifuging the UCB/ HES mixture in the original collection blood bag (50g for 5 min at 10°C). The leukocyte-rich supernatant is expressed from the bag into a 150-ml plasma transfer bag and centrifuged (400g for 10 min) to sediment the cells. Finally, the sedimented leukocytes are resuspended in supernatant plasma to a total volume of 20 ml and are then designated as leukocyte concentrates. The transfers can be done in a closed system, or the bags may be connected by the standard spike connectors with the usual precautions to prevent bacterial and fungal contamination.53

**Cryopreservation or cryoprotectants:**

Cryopreservation is used to prevent dehydration of cord blood, by inhibiting the increased concentration of sodium that can occur during ice formation and by decreasing the amount of water absorbed into ice crystals at any given temperature. Examples are Dimethyl sulfoxide
(DMSO), Hydroxyethyl starch (HES), protein. All can be used together to improve HSC survival.

Dimethyl sulfoxide (DMSO) is used at a final concentration of 10% (vol/vol). The required volume of sterile, chilled DMSO solution is added to the blood bag over the course of 15 minutes by using a syringe pump and an orbital mixer to assure smooth but vigorous mixing. Cryoprotectants and UCB or Leukocyte concentrates are kept cold with wet ice throughout the addition. When the concentration of DMSO reaches 10%, cell suspensions are transferred to Cryocyte freezing containers, placed in aluminum canisters, and then deposited horizontally on a level surface inside a -80°C freezer. When the temperatures are below -50°C, the units are transferred to the liquid phase of a liquid Nitrogen freezer for storage.\textsuperscript{53, 54}

**Thawing Units after Storage in Liquid Nitrogen.** To thaw the unit for transfusion, the bag is lifted to the gas phase of liquid nitrogen for 15 minutes and then exposed to ambient air for 5 min to allow the plastic to regain some elasticity. The bag is then immersed in 37°C water for thawing as rapidly as possible.\textsuperscript{53, 54}

**Removal of Hypertonic Cryoprotectant.** Immediately after being thawed, each UCB unit is diluted with an equal volume of a solution containing 2.5% (wt/vol) human albumin and 5% (wt/vol) Dextran 40 in isotonic salt solution, with continuous mixing, and then centrifuged at 400g for 10 min. The supernatant is removed, and the sedimented cells are resuspended slowly in fresh albumin/dextran solution to a volume appropriate for infusion to patients.\textsuperscript{52}

However, if prompt processing after collection is not possible within one hour of collection, cord blood should be stored at 4°C to maximally preserve cell viability. HSC stored at 4°C show a progressive loss of nucleated cells, cell viability and CD34+ cells. Cord blood processing could be delayed for as long as 72 hours after collection, provided samples are stored at 4°C.\textsuperscript{53, 55, 56}
Factors that may influence the suitability of UCB units are,

1. Birth weight- Birth weight is the main factor influencing total nucleated cells (TNC) CD34 cells, and colony-forming unit (CFU). Studies have showed that each 500 g increase in birth weight contributed 11% increase in total nucleated count, a 28% increase in CD34+ cell counts and a 22% increase in CFU-GM. Birth weight >3500g could give optimal values of TNC, CD34+ cell and CFU.30,56,57

2. Gestation age- Gestational age greater than or equal to 37 weeks and less than or equal to 40 weeks would provide optimal levels of CD34 cells, CFU-GM and TNC. However, the CFU and CD34+ cell counts dropped with gestational age, suggesting a loss of haematopoietic potential with longer gestational age.30,56

3. Placental weight- Placental weight greater than 600 grams produced a better volume and increased levels of TNC.4,57

4. Cord clamping time- Early cord blood clamping done between 15-30 seconds following delivery of the baby may increase the volume of collection.4,56

5. Mode of delivery- Caesarean section produced higher volume and reduced white blood cell count compared to vaginal delivery.30,56

UCB is now an alternative source for hematopoietic stem cells for transplantation to BM.11,12,30,52,58

CORD BLOOD STEM CELL TRANSPLANTATION

Umbilical cord blood is a suitable source of Haematopoietic stem cell for haematopoietic reconstitution.30,48,49,59 It is being used clinically for transplantation therapy in children and adults with a wide variety of malignant and non-malignant diseases and other diseases as an alternative to mismatched related or matched unrelated bone marrow or peripheral blood haematopoietic stem cell transplantation.4,59,60
Compared to peripheral blood and bone marrow, UCB has several advantages making it an attractive alternative source of haematopoietic stem cells.\(^2,61\)

1. Absence of risk to mother and child.\(^{11,52,61,62}\)

2. Availability – UCB unlike BM is easier to obtain and rapidly accessible and stored, thus it is readily available from CB banks.\(^5,63\)

3. Available evidence suggests that UCBT may be performed with greater degrees of HLA mismatch than for unrelated donor BMT which increases the pool of potential transplant donors.\(^2\) Donors CB units should have at least 4/6 HLA matching at – A, -B and DRB1 loci against BMT in which the HLA is 6/6. Minimal cell dose infused in UCBT is \(>2 \times 10^7\) NC/kg with optimum CD34 positive cells of \(1.7 \times 10^5\) body weight.\(^6,17,61,64\)

4. Graft versus host disease – the incidence of both acute and chronic GVHD are lower in patients with CBT, despite the HLA disparity. The lower incidence of GvHD may be explained by the lower number and mostly naïve repertoire of CB derived T-cells.\(^52,61,64\)

5. Transmission of infection – using appropriate methods of screening, the risk of transmission of infectious agents (bacteria, fungi, virus such as cytomegalovirus Epstein – Barr virus, HIV & Hepatitis B) appears to be low in UCB\(^4\). Also, there is a low rate of transmission of genetic diseases\(^30,52,61\).

6. Easier targeting of ethnic minorities and an increased pool of rare haplotypes.\(^31,50\)

**Limitations of umbilical cord blood transplant**

Despite the potential advantages, UCBT has a number of limitations
1. Limited volume: the limited number of cells collected in units of CB from a single donor, implies that one cannot always get enough cells from a single CB donor to be able to successfully engraft an adult or high weight child.11,61

2. Engraftment: HSC transplanted in UCB, is 10 times less than BM transplants. This leads to greater incidence of graft failure and prolonged time for engraftment i.e. the time for neutrophils and platelet engraftment is slow thereby leading to a longer hospital stay.11, 16, 59, 64.

3. There is also the possibility of transfer of genetically abnormal cells.4

**Related studies on the suitability of cord blood for HSCT**

Broxmeyer and colleagues in 1989 studied human umbilical cord blood as source of haematopoietic stem cells and concluded that umbilical cord blood contains sufficient number of HSC and progenitor cells for both allogeneic and autologous stem cell transplantation.11

Gluckman et al12 in 1988 performed the world’s first UCBT for a child with Fanconi’s anaemia using cord blood from an HLA identical sibling.

Vomels et al65 in 1993 published a case report of a four-year boy with an X-linked lymphoproliferative disease that was treated with transplantation of cord blood cells from an HLA identical sibling.

In 1995, Issaragrisil et al66 in a brief report presented a two and half-year old girl with αβ-thalassemia who was treated with cord blood transplantation from HLA – identical brother.

Rubinstein et al67 in 1998 studied the outcomes of umbilical cord blood transplants from unrelated donors. The study revealed a low rate of neutrophil and platelet engraftments, and
that the slow neutrophil engraftment was associated with the leukocyte content of the graft, while transplantation-related events were associated with the patient's underlying disease, age, the number of leukocytes in the graft, the degree of HLA disparity, and the transplantation centre. However, they concluded that placental blood is a useful source of allogeneic haematopoietic stem cells for bone marrow reconstitution.

In another study, Gluckman et al. did a 9-year follow up study on 45 transplant centres. The study was based on a prospective assessment of the outcome of cord blood transplantation from related and un-related UCB donors. Their findings suggested that younger age, lower weight and cytomegalovirus negative serologic results are favorable prognostic factors. They concluded that cord blood is a feasible alternative source of haematopoietic stem cells for paediatric and some adult patients with major haematologic disorders, particularly if the donor and the recipient are related.

Roucha et al. did a seven-year study to assess graft versus host disease in children who have received cord blood or BM transplantation from an HLA identical sibling. The study comprised of 113 recipients of UCB and 2052 recipients of BM from HLA –identical siblings, for the treatment of either malignant or non-malignant diseases. There was a lower incidence of acute and chronic GVHD with recipients of UCBT than with recipients of BMT. The authors found that the risk of acute and chronic GVHD was lower with children who received cord blood transplantation compared to those who received BMT.

Dalle et al. studied 64 transplant patients, of which 36 were HLA- mismatched CB and 28 HLA matched BM, with a nucleated cell dose over 2.5 x 10^7 cells/kg. Three year survival was 59% in CB and 57% in BM patients, thus they concluded that CB as a source of stem cells offers a graft to almost every child of an unrelated transplantation, with a survival similar to that of related BMT.
Following the benefits of UCBT for paediatric patients, many clinical studies have focused on the adoption of UCB transplantation for the treatment of adult malignancies. A major concern regarding adult patients is the restricted number of HSC in the UCB graft.\textsuperscript{6} To overcome the low cell content of single UCB units, various alternatives have been used such as co-infusion of a related umbilical cord blood graft and a haploidentical or third party donor peripheral blood graft and double umbilical cord blood transplantation (dUCBT).\textsuperscript{6,57}

Barker \textit{et al}\textsuperscript{15} in 2005 studied the dUCBT on twenty-three adult patients with haematological malignancies. The aim was to determine the safety of the combined transplantation of two partially human leukocyte antigen (HLA)-matched UCB units. They reported that the disease-free survival was 57\% at 1 year and 72\% of patients alive if they received transplants while in remission. They therefore concluded that, transplantation of two partially HLA-matched UCB units is safe, and may overcome the cell-dose barrier that limits the use of UCB in many adults and adolescents. Similarly, Brunstein \textit{et al}\textsuperscript{70} in 2007 investigated adult patients with haematological malignancy who received dUCBT, their work revealed 38\% event-free survival and 45\% survival after 3 years. Their findings supported the use of dUCB for extending the availability of transplantation therapy, particularly for older patients. Long \textit{et al}\textsuperscript{71} carried out a similar research on unrelated UCBT in adult patients with high-risk disease. Their studies showed 19\% survival after 3 years. Their study suggested that unrelated umbilical cord blood transplantation is a viable option for adult patients and should be explored in patients with early-stage disease.

Wagner \textit{et al}\textsuperscript{17} in 2002 studied the influence of CD34+ cells on recipients of UCBT from related and unrelated donors. The aim was to investigate the role of CD34+ cell dose on the rate of engraftment, treatment related mortality and survival of patients treated for malignant and non-malignant diseases. Their work suggested that the rate of engraftment, treatment
related mortality and survival was based on CD34+ cell dose (1.7 X 10^5 CD34+ cell /Kg). Mahantappa and colleagues in 2007 in India, conducted a study to show the correlation between the volumes of cord blood collected, total nucleated cells harvested, and CD34+ cells count. Their work showed that the volume of cord blood collected and total nucleated cells harvested did not have any correlation with CD34 cells present. They concluded that suitability of UCB sample for transplantation should be based on the number of CD34+ cells (1.7 X 10^5 CD34 stem cells per kilogram body weight) instead of volume of CB collected.

However, adopting this technique in our environment, it will be worthwhile estimating the level of CD34+ cells and total nucleated cell in UCB, thereby providing local data. In addition, an attempt to extend the outcome of this research as a way to enhancing HSCT in Nigeria may yield interesting and beneficial results.

With the 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 to transplant centres. Currently, more than 6,000 cord blood transplantations have been performed worldwide.

Dhot and colleagues in 2003 conducted a study on collection, separation, enumeration and cryopreservation of 30 samples of cord blood HSC. The samples were collected after delivery of infants prior to expulsion of placenta. The average cord blood volume collected was 101.33 ml; total nucleated cell counts ranged from 4.8-27.2 X 10^7/ml. Viability count of nucleated cells was 98.4%. After 6 months of cryopreservation, the viability count on revival was over 82.1%. The authors concluded that with UCB, donors could easily be identified for any given patient who needs a transplant.
M-Reboredo and colleagues\textsuperscript{73} in 2000 in Spain conducted a study to establish and standardize a method for unrelated cord blood banking as well as the biological characterization of the samples (UCB). They had 938 UCB units collected into a modified triple bag system and 6\% hydroxyethyl starch was added to induce red blood cell agglutination. The UCB was separated by two centrifugation steps into three components: buffy coat, red cell and plasma fractions. The mean volume collected was 84.6 ± 23.6 ml (range 45-172 ml), mean total nucleated cell count was 0.90 ± 0.37 x 10\(^9\)/ml (range 0.43-2.47 x 10\(^9\)/ml) and the average number of CD34\(^+\) cells was 2.46 ± 2.72 x10\(^6\)/ml (range 0.2-36.6x10\(^6\)/ml) with an average number of 79 ± 72 x 10\(^4\)/ml CFU-GM (range 1.4-880x 10\(^4\)/ml). All samples were 99.9\% viable before cryopreservation. Their study suggested that the NC counts significantly correlated with the volume collected, number of CD34\(^+\) cells and CFU-GM.

Keersmaekers \textit{et al}\textsuperscript{14} in 2004 conducted a study on factors affecting umbilical cord blood stem cell suitability for transplantation in an in utero collection program. Their results reviewed that gestational age, infant race, parity, birth weight, and infant sex are predictors of acceptable banking TNC count of at least 90 × 10\(^7\)/ml.

Ballen \textit{et al}\textsuperscript{74} studied the factors that determine the optimal cord blood units based on maternal and neonatal parameters. Their results showed mean volume of 106 ml (range of 75 - 218 ml), mean total nucleated cell (TNC) count was 94 x10\(^7\)/ml (range 25-348), mean total CFU-GM 2.3 x 10\(^6\)/ml (range 0.04-39.6) and mean total CD34\(^+\) cell count of 3.1 x 10\(^6\)/ml (range 0.07-25.2). The mean viability was 95\% (range 70-100\%). Their work revealed that the volume of cord blood collected was strongly correlated with CD34\(^+\) cell counts, CFU-GM, TNC count and viability. Their finding suggested that, cord blood units should be selected without regard to race, maternal age, or sex of the baby and optimal results.
would be obtained by selecting first or second babies, babies with a gestational age less than or equal to 40 weeks, and weight >3600 g.

In Nigeria, Idemudia and Bazuaye\textsuperscript{25} conducted a study on the biochemical parameters of cord blood with a view to assess its suitability for stem cell transplantation. The biochemical parameters were analyzed and hyperkalaemia was observed. They concluded that UCB could become an important source of stem cell in sub-sahara Africa especially with the large number of deliveries. Careful selection of quality cord blood must be enforced to avoid contaminants and haemolysis, which may be responsible for the hyperkalaemia as seen in the study.
CHAPTER THREE

SUBJECTS AND METHODS

STUDY DESIGN

The study was a cross-sectional study.

STUDY AREA

This study was carried out in the departments of Haematology and Obstetrics at the University of Benin Teaching Hospital (UBTH), Benin City, Edo State. The hospital is a tertiary health institution located along the Lagos-Benin express way in Egor Local Government Area, Benin City, Edo State, Nigeria. It has over 600 patient beds, with several specialties. It serves as a major referral centre to the surrounding states of Delta, Kogi, Ondo and other states like Anambra. However, it also has the only stem cell transplant centre in Nigeria, East, West, and Central Africa.

STUDY POPULATION

The population were pregnant women who booked, received antenatal care and delivered in the labor room or labour ward theatre of the Department of Obstetrics, University of Benin Teaching Hospital.

SAMPLE SIZE ESTIMATION

The sample size was calculated from the formula for a cross sectional study:

\[ n = \frac{z^2pq}{d^2} \]

Where \( n \) = minimum sample size.

\( z \) = standard deviation (1.96)
p= proportion of viable stem cell in the umbilical cord was 0.98 (being a proportion found in previous study). 

q = 1-p

d= degree of precision (0.05) attrition

Mathematically, sample size (n) is = \((1.96)^2 \times 0.98 \times (1-0.98) / (0.05)^2\)

\[ n = 30 \]

10% of non-viable cell (attrition) = 3

The working sample size for this research was 33

**SAMPLING TECHNIQUE**

Patients presenting at the labour ward and patients for elective caesarean section who met the inclusion criteria were recruited consecutively.

**INCLUSION CRITERIA**

**Mother**

- Booked pregnant woman with uneventful index antenatal history who gave consent for the study

**Neonate**

- Term baby (37–42 weeks)
- Babies with normal birth weight (2.5–4.0 Kg)
EXCLUSION CRITERIA

Mother
- Age less than 18 because the parents will be required to give consent and above 40 years may be associated with chromosomal abnormalities.
- Diseases complicating pregnancy: Pregnancy induced hypertension (PIH), eclampsia, diabetes (gestational or insulin dependent), co-existing heart, kidney or lung disease, disseminated intravascular coagulation (DIC), haemoglobinopathies (Sickle cell disease) are usually excluded as donors of stem cells for transplant due to possibility of altered cord blood parameters.
- Drug or alcohol addicts are usually known to be associated with genetic abnormalities,
- Mothers with Infectious diseases such as HIV, hepatitis, syphilis, are usually excluded from donating stem cells for transplant

Neonate
- Hydrops foetalis
- Obvious congenital/chromosomal abnormality

The placenta of the above babies are not fit to donate stem cells for transplant and also there is the possibility that the appropriate yield might not be obtained.

ETHICAL CONSIDERATIONS

Ethical approval from the hospital’s ethics and research committee was obtained for the study. Written informed consent was obtained from mothers.

DATA COLLECTION

Data on demographics, history of previous haematologic, cardiovascular, endocrine, metabolic or neurological diseases, cigarette smoking, alcohol and drug history as well as
events of antenatal period were obtained by means of an interviewer-administered questionnaire as well as from paricipants’ case notes.

All the newborns were examined physically to exclude those with gross congenital malformation. The details of the delivery, birth weight and placenta weight were documented.

SAMPLE COLLECTION AND STORAGE

Cord blood collections were performed after the delivery of the foetus, the umbilical cord was clamped and ligated before cutting it 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 (4ml) were collected from the umbilical cord vein.

Three and a half millilitres (3.5ml) 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 temperature of 4°C and were transported to Ladoke Akintola University Teaching Hospital (LAUTECH), where they were assayed for total nucleated cell, viability count and CD34+ cells, within 24 hours of collections. Samples were analyzed in batches.

1. ENUMERATION OF CD34+ CELLS

CD34/PE kit

CD34/PE Kit from Sysmex Partec Germany,

Ref-05-7-166IP,

Lot number –E1711KH,

Expiry date- 08/2017.
CD34/PE (phycoeryrin-conjugated) is a monoclonal antibody coupled with the fluorophore PE and intended for staining of human CD34 cells and subsequently analysis on flow cytometers

**Principle:**
The CD34⁺ cell count was assessed using flow cytometric method on Cyflow cube 6, Sysmex Partec, Germany. The Cyflow instrument is a compact cell counter with a built-in computer. The basic design involves four major elements: optics, fluidics, electronics and computer with specific software for the type of cell to be counted. Cyflow cube 6 is equipped with blue laser light excitation (488nm), capable of analyzing forward scatter (FSC), side scatter (SSC) and orange fluorescence detecting parameter.

CD34/PE (phycoeryrin-conjugated) is a monoclonal antibody coupled with the fluorophore PE and intended for staining of human CD34 cells. The antibody 4H11(APG) reacts with class 111 epitope on CD34, a 111-115 kDa surface protein, which is present on many stem cell populations.

Storage and preparation of reagents, samples and standards were done according to the manufacturer’s instructions.

**Reagents**
CD34/PE, 7-AAD, Human Erythrocyte lysing reagent.

**Methods**
1. A 100 uL sample of anticoagulated blood was added to 20 uL of CD34/PE into a test tube.
2. The mixture was mixed thoroughly.
3. It was incubated for 15 minutes in the dark at room temperature.
4. A 100 uL of 7AAD was added to the mixture and thoroughly mixed.

5. The mixture was then incubated in the dark at room temperature for 10 minutes.

6. A 2.5ml of erythrocyte lysing was added to the mixture and thoroughly mixed.

7. This was incubated for 20 minutes in the dark at room temperature.

8. The sample mixture was analyzed on Cyflow cube 6 and the events was displayed in a plot of side light scatter versus forward light scatter.

9. Gating was set around CD34+ cells and orange fluorescence associated with these events was displayed as a single-parameter histogram of number.

   Minimal acceptable value is $2 \times 10^6$/ml.

2. **VIABILITY COUNT**

   7-Aminoactinomycin D (7-AAD) dye, from Sysmex Partec Germany,

   Lot number –14111,

   Expiry date- 01/2017.

   7-Aminoactinomycin D (7-AAD) is an in vitro stain for the quantitative measurement of viable and dead cell in whole blood using flow cytometry.

   **Principle:**

   7-AminoactinomycinD (7-AAD) is a dye (molecular weight 1270) that preferentially binds to Guanine-Cytosine rich (G-C) regions of DNA. It is usually excluded from live cells and can be used to label the DNA of dead cells in flow cytometry.

   **Reagents**

   7-AAD solution and Erythrocyte Lysing Reagent.

   **Equipment**

   Cyflow cube 6, Pipette tips and pipette, Test tube, Stop clock.
Procedure

1. A 100ul of whole blood was put into a test tube and 100ul of 7AAD was added.
2. The mixture was thoroughly mixed and incubated for 10 minutes in the dark at room temperature.
3. A 2.5 ml of erythrocyte lysing reagent was added to the mixture and was gently shaken.
4. The mixture was incubated for 20 minutes in the dark at room temperature.
5. 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.
6. Gating was set around viable cells and green fluorescence associated with these events was displayed as a single-parameter histogram of number.

Acceptable value for viability count = 75-100%

3. ESTIMATION OF TOTAL NUCLEATED CELLS

Principle

It is based on aperture impedance method which enumerates cells in a small aperture by measuring changes in electrical resistance as the cell passes through the orifice. A constant current passes between two platinum electrodes on either side of the orifice. The diluent that suspends the cells is more electrically conductive than are the cells. Hence, as each cell passes through the orifice, there is a momentary decrease in electrical conductance so that an electrical impulse is generated and recorded electronically. The drop in voltage is proportional to cell size, allowing average cell size to be determined simultaneously.
**Procedure**

1. The haematological parameters were analyzed using the Sysmex haematology autoanalyser model KX21N, Japan.
2. EDTA anticoagulated samples were mixed continuously on a mixer until analysed.
3. Samples were consecutively placed in the receiver of the autoanalyser, which aspirated 20µL of blood from the sample.
4. Cell count was automatically done by the machine and the result printed out.

These parameters include: Total Nucleated cells and other haematological parameters of cord blood.

- Minimal value for TNC for children = $1.77 \times 10^7$/kg
- Minimal value for TNC for adult = $2.0 \times 10^7$/kg

**DATA ANALYSIS:**

Data was presented in tables and analysed using the SPSS 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.
CHAPTER FOUR

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. While the seven other samples were stored and analysed after twenty-four hours, these analysis was done in order to show the effect of prolonged storage (24 hours) on total nucleated cells, CD34+ cells and viability count.

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 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, twenty five (75.8%) were between the range 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 500grams, twenty-three (69.7%) weighed between 500-600grams, 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 -3.90kg while two (6.1%) were greater than 3.90kg
### Table 1A: Demographic Parameters of Maternal and Neonatal Factors

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

Key: CS - Caesarean Section  
SVD - Spontaneous Vaginal Delivery.
Table 1B: Mean and Range Distribution of Maternal Age, Parity, Gestational Age, Placental Weight and Birth Weight.

<table>
<thead>
<tr>
<th>Maternal and Neonatal Factors</th>
<th>n</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (year)</td>
<td>33</td>
<td>32.0 ± 4.40</td>
<td>23.0 - 40.0</td>
</tr>
<tr>
<td>Parity</td>
<td>33</td>
<td>3.18 ± 1.91</td>
<td>1–9</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>33</td>
<td>38.84 ± 1.09</td>
<td>37–41</td>
</tr>
<tr>
<td>Placental weight(grams)</td>
<td>33</td>
<td>548.48 ± 71.20</td>
<td>400–650</td>
</tr>
<tr>
<td>Birth weight (kilogram)</td>
<td>33</td>
<td>3.29 ± 0.42</td>
<td>2.50 -4.00</td>
</tr>
</tbody>
</table>
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 was 3.89 x 10^4 ± 1.48 cells/ml with a range of 2.00 - 6.99 x 10^4 cells/ml. The mean viability of the cells was 90.00 ± 9.55% with a range of 60.0 - 98.20%.

Table 2: Mean and Range Distribution of CD34+cells, TNC, Viability Count and Processing Time

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNC(x10^6 cells/ml)</td>
<td>11.14 ± 4.64</td>
<td>2.90 - 21.10</td>
</tr>
<tr>
<td>CD34+( x 10^4 cells/ml)</td>
<td>3.89 ± 1.48</td>
<td>2.00 - 6.99</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>90.0 ± 9.55</td>
<td>60.0 - 98.20</td>
</tr>
<tr>
<td>PT (hours)</td>
<td>9.60 ± 4.47</td>
<td>5.00 - 22.0</td>
</tr>
</tbody>
</table>
Table 3 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).

TNC count increases with increasing birth weight however the increment 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 cesarian 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 3: Comparing the Mean Distribution of TNC (X10⁶/ml) with Maternal Age, Parity, Gestational Age, Mode of Delivery, Gender, Birth Weight and Placental Weight.

<table>
<thead>
<tr>
<th>Neonatal and maternal factors</th>
<th>TNC x10⁶/ml</th>
<th>F value</th>
<th>T value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>13.40 ± 0.00</td>
<td>2.353</td>
<td></td>
<td>0.112</td>
</tr>
<tr>
<td>25-35</td>
<td>11.90 ± 4.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;35</td>
<td>7.93 ± 4.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15.60 ± 2.05</td>
<td>6.161</td>
<td></td>
<td>0.006**</td>
</tr>
<tr>
<td>2-5</td>
<td>12.60 ± 5.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>8.90 ± 3.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5-3.9</td>
<td>11.03 ± 4.70</td>
<td></td>
<td>-0.533</td>
<td>0.598</td>
</tr>
<tr>
<td>&gt;3.9</td>
<td>12.85 ± 3.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 – 39</td>
<td>10.80 ± 3.89</td>
<td></td>
<td>-0.472</td>
<td>0.640</td>
</tr>
<tr>
<td>&gt;39</td>
<td>11.60 ± 5.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>10.91 ± 4.32</td>
<td></td>
<td>-0.290</td>
<td>0.774</td>
</tr>
<tr>
<td>Male</td>
<td>11.38 ± 5.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>10.91 ± 4.81</td>
<td></td>
<td>-0.188</td>
<td>0.852</td>
</tr>
<tr>
<td>SVD</td>
<td>11.20 ± 4.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental weight (grams)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;500</td>
<td>9.96±3.28</td>
<td>0.984</td>
<td></td>
<td>0.385</td>
</tr>
<tr>
<td>500-600</td>
<td>10.83±4.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;600</td>
<td>13.7±6.34</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key; **p-value<0.05, statistically significant.
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 increment 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 10^4 vs 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 cesarian 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 count tends to decline with increasing gestational weight. However this was not statistically significant (p=0.783).
Table 4: Comparing the Mean Distribution of CD34+ Cells (X 10⁴/ml) with Maternal Age, Parity, Gestational Age, Mode of Delivery, Gender, Birth Weight and Placental Weight.

<table>
<thead>
<tr>
<th>Neonatal and maternal factors</th>
<th>CD34+ cells x 10⁴/ml</th>
<th>F value</th>
<th>T value</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>5.30 ± 0.00</td>
<td>0.650</td>
<td></td>
<td>0.529</td>
</tr>
<tr>
<td>25-35</td>
<td>3.94 ± 1.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;35</td>
<td>3.54 ± 1.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.24 ± 1.03</td>
<td>5.988</td>
<td></td>
<td>0.006**</td>
</tr>
<tr>
<td>2-5</td>
<td>4.37 ± 1.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>3.19 ± 1.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5-3.9</td>
<td>3.87 ± 1.49</td>
<td>-0.314</td>
<td></td>
<td>0.756</td>
</tr>
<tr>
<td>&gt;3.9</td>
<td>4.21 ± 1.82</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA (weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37 – 39</td>
<td>3.95 ± 1.47</td>
<td>0.277</td>
<td></td>
<td>0.783</td>
</tr>
<tr>
<td>&gt;39</td>
<td>3.80 ± 1.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3.50 ± 1.79</td>
<td>-0.263</td>
<td></td>
<td>0.794</td>
</tr>
<tr>
<td>Male</td>
<td>3.61 ± 2.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>3.46 ±1.15</td>
<td>-1.173</td>
<td></td>
<td>0.250</td>
</tr>
<tr>
<td>SVD</td>
<td>4.10 ±1.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental weight (grams)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;500</td>
<td>3.54 ±1.46</td>
<td>1.229</td>
<td></td>
<td>0.307</td>
</tr>
<tr>
<td>500-600</td>
<td>3.76 ±1.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;600</td>
<td>4.82 ±1.80</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key; **p-values<0.05, statistically significant.
The bar chart (1a-1g) below shows the mean values of TNC and CD34\(^+\) cells with maternal and neonatal factors.

Figure 1a: showing the mean value of CD34\(^+\) Cells and TNC with maternal age
Figure 1b: showing the mean value of CD34+ Cells and TNC with parity

Figure 1c: showing the mean value of CD34+ Cells and TNC with birth weigh
Figure 1d: showing the mean value of CD34⁺ Cells and TNC with mode of delivery.

Figure 1e: showing the mean value of CD34⁺ Cells and TNC with Gestational Age (weeks)
Figure 1f: showing the mean value of CD34+ Cells and TNC with Placental weight (grams)

Figure 1g: showing the mean value of CD34+ Cells and TNC with Gender.
Table 5a, 5b and 5c shows the compared mean values of TNC count, CD 34+ cells and Viability time with storage time respectively. TNC count and CD34+ cells decrease with prolonged storage time (greater than 24 hours) and was statistically significant (p = 0.00). Similarly viability count tend to decline progressively with increasing storage time (greater than 24 hours) and they were statistically significant (p = 0.02).

**Table 5a: Comparing the Mean Distribution of TNC with Storage Time**

<table>
<thead>
<tr>
<th>Storage time</th>
<th>N</th>
<th>Mean ± SD of TNC x10^6/ml</th>
<th>F values</th>
<th>T values</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24 hours</td>
<td>33</td>
<td>11.14 ± 4.64</td>
<td>16.50</td>
<td>4.81</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;24 hours</td>
<td>7</td>
<td>2.60 ± 0.30</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5b: Comparing the Mean Distribution of CD 34+ Cells with Storage Time.**

<table>
<thead>
<tr>
<th>Storage duration(hrs)</th>
<th>N</th>
<th>Mean ± SD of CD34+ cells.</th>
<th>F values</th>
<th>T values</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24</td>
<td>33</td>
<td>3.89 ± 1.48</td>
<td>15.89</td>
<td>6.602</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;24</td>
<td>7</td>
<td>0.15 ± 0.174</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5c: Comparing the Mean Distribution of Viability Count with Storage Time.

<table>
<thead>
<tr>
<th>Storage duration</th>
<th>N</th>
<th>Mean ± SD of Viability count (%)</th>
<th>F values</th>
<th>T values</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 24 hours</td>
<td>33</td>
<td>90.00 ± 9.55</td>
<td>6.41</td>
<td>12.28</td>
<td>0.016</td>
</tr>
<tr>
<td>&gt;24 hours</td>
<td>7</td>
<td>44.70 ± 0.99</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The bar chart below shows the mean value of TNC, CD34+ Cells, and Viability count with storage time (hours).

Figure 2: showing the mean value of TNC (ml), CD34+ Cells (ml), and Viability count (%) with storage time (hours).
Table 6 shows the correlation between TNC, CD34+ cells, viability and processing time (PT). A very strong positive correlation was found between TNC and CD34+ cells ($r=0.760, P = 0.000$) as in figure 3. A 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 figure 4.

**Table 6: Correlation Between TNC, CD34+ cells, Viability Count and Processing Time (PT)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>TNC</th>
<th>CD34+ cells</th>
<th>Viability</th>
<th>PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNC</td>
<td>r</td>
<td>1.00</td>
<td>0.760</td>
<td>0.475</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>-</td>
<td>0.000</td>
<td>0.005</td>
</tr>
<tr>
<td>CD34+ cells</td>
<td>r</td>
<td>0.760</td>
<td>1.00</td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.000</td>
<td>-</td>
<td>0.278</td>
</tr>
<tr>
<td>Viability</td>
<td>r</td>
<td>0.475</td>
<td>0.195</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.005</td>
<td>0.278</td>
<td>-</td>
</tr>
<tr>
<td>PT</td>
<td>r</td>
<td>-0.505</td>
<td>-0.197</td>
<td>-0.859</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.003</td>
<td>0.272</td>
<td>0.000</td>
</tr>
</tbody>
</table>
The graph below shows positive correlation between CD34+ cells and TNC.

Figure 3: Scatter gram of correlation between CD34+ cells and TNC.

The graph below shows negative correlation between Viability count and PT.

Figure 4: Scatter gram of Viability Count and Processing Time
Correlations of TNC with Maternal and Neonatal Parameters.

Weak positive correlations was observed between birth weight, gender, mode of delivery, placental weight with TNC and they were not statistically significant but a very weak negative correlation was found with gestational age ($r = -0.020$) and it was statistically not significant ($p = 0.913$) as shown in table 7 however parity and maternal age had a negative correlation with TNC that was statistically significant ($r = -0.539; p = 0.001$ and $r = -0.358; p = 0.041$).
Table 7: Correlation of TNC with Maternal Age, Parity, Gestational Age, Mode of Delivery, Gender, Birth Weight and Placental Weight.

<table>
<thead>
<tr>
<th>Maternal and Neonatal Parameters</th>
<th>TNC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td>Parity</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td>Gestational age</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td>Gender</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td>Birth weight</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td>Placental weight</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
</tbody>
</table>

Key;

r = Pearson correlation

**p-values <0.05, statistically significant.
Correlations of CD34+ Cells with Maternal and Neonatal Parameters

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 8.
Table 8: Correlation of CD34+ cells with Maternal Age, Parity, Gestational Age, Mode of Delivery, Gender, Birth Weight and Placental Weight.

<table>
<thead>
<tr>
<th>Maternal and Neonatal Parameters</th>
<th>CD34+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>-0.177</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.325</td>
</tr>
<tr>
<td>Parity</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>-0.532</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.001**</td>
</tr>
<tr>
<td>Gestational age</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.785</td>
</tr>
<tr>
<td>Gender</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.794</td>
</tr>
<tr>
<td>Birth weight</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.756</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>0.206</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.250</td>
</tr>
<tr>
<td>Placental weight</td>
<td>r</td>
</tr>
<tr>
<td></td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>0.176</td>
</tr>
</tbody>
</table>

Key;

r = Pearson correlation

**p-values <0.05, statistically significant.
CHAPTER FIVE

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 predict engraftment. This study was to demonstrate 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.

In this study it was observed that the average CD34+ cells was $3.89 \pm 1.48 \times 10^4$/ml, with a range of 2.0 - 6.99 x 10^4 cells /ml. Report from Kristin et al\textsuperscript{76} in the United State of America showed a mean value of CD34+cells of $3.91 \pm 2.21 \times 10^4$/ml and a range of 0.20 - 15.32 x10^4/ml. While Mihaela et al\textsuperscript{77} in Rome reported higher mean value of CD34+ cells to be $4.83 \pm 0.82 \times 10^4$/ml, with a range 3.12 - 6.30 x 10^4/ml. This higher mean value could be due to large sample size analyzed by Mihaela et al.\textsuperscript{77} However, the value of CD34+ cells found in this study was within the mean value as reported in other studies.

The TNC count in this study had an average value of $11.14 \pm 4.47 \times 10^6$ cells/ml, with a range of 2.0 – 21.0 x 10^6/ml. Studies conducted by M-Reboredo et al in Spain and Mutlak in India, reported a mean value of 10.51 x 10^6/ml and 9.88 x 10^6/ml respectively.\textsuperscript{73,78} However Kristin et al in America and Mihaela et al in Rome showed a higher mean value of TNC to be 13.46 x 10^6 and 12.74 x 10^6/ml respectively. Some studies have shown that Caucasian infants had a higher number per ml of CFU, CD34+ cells, TNC counts compared to African American infants.\textsuperscript{78} This probably explains the lower mean value observed in this study. The finding in this study is within the range found in Spain and India.\textsuperscript{74,78} 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 higher than found in Africans. This probably could be due to geographical locations, acute and chronic malaria infection, medicinal herbs consumption and poverty with malnutrition in African countries. TNC has emerged as the most commonly reported parameter in addition to cord blood volume in estimating 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.

The number of TNC and CD34\(^+\) cells is used as dependent factor for selecting UCB units for transplantation. This study showed a significant positive correlation between TNC and CD34+ cells. This finding is in keeping with other studies. Chandra \textit{et al} \cite{chandra} reported that TNC correlated positively with CD34+ cells in cord blood. The significant of positive correlation between CD34+ cells and TNC has enabled some centres to use TNC to assess the quality of the transfused stem cells in centres where the facility for 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.

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 values found in previous studies. Report from M-Reboredo \textit{et al}, Ballen \textit{et al} and Dhot and colleagues\cite{reboredo,ballen,dhot} observed a mean value of 98.4\%. 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 (200km). It was observed that the median processing time in this study was 10 hours. Some authors have observed that samples processed more than 10 hours from collection have significant lower values of viability count, TNC and CD34\(^+\) cells.\cite{viability}
Processing time of the samples had a negative correlation with viability count, TNC and CD34+ cells; however, CD34+ cells were not significantly affected, but viability count and TNC were affected. This is in agreement with other researchers' findings. Isidro et al. reported that viability count was reduced in samples that were processed at a longer time. Isidro 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 were 8.54 x 10^6/ml, 3.74 x 10^4/ml and 45.5% respectively. Previous studies have showed that the numbers of TNC, CD34+ cells and viability count correlate well with outcome, thus most cord blood with higher unit of TNC count and viability count are used for transplant in order to have a good outcome. It is therefore essential to maintain the viability of cells during transport and storage at temperatures of 1-8°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. Interaction between temperature and storage time may influence the quality of HSC. Cord blood with low TNC, CD34+ cells and viability count are rejected by cord blood bank. Previous literatures have showed that there is no absolute values for TNC and CD34+ cells, hence the values given by various laboratories are not comparable, however 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 and viability count greater than 75%. From this analysis the average value was 11.14 x10^6 of TNC/ml, therefore if 100ml UCB volume is collected for HSCT, then 11.14 x10^8 cell (11.14x10^6 x100 ml=111.4 x10^7 cells) can be obtained in that particular unit, which will be adequate for a patient weighing between 45 and 55kg body weight. Despite the fact that the Caucasian values of TNC are higher than that of African
illustration above, shows that the values obtained for TNC in this study is above minimum value recommend by World Marrow Donor Association. In this study the mean cord blood cell values of \(11.14 \times 10^6\) (4.80 - 21.10) TNC, \(3.89 \times 10^4\) (2.0 - 6.99) of CD34+ cells and viability of 90\% (60-98.2\%) were found to be within the reported mean values for transplant, however the dose received is measured per recipient body weight and the volume of the harvest is a strong determinant but this is not the focus of the index study.

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 maternal age and maternal 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.\(^{54,74}\)

A previous report showed that women older than 25 years of age carry more TNCs, and younger maternal age was associated with a higher CD34+ cell concentration.\(^{55}\) However in this study the younger mothers 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 age.\(^{85}\)

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 significantly higher. Similarly, a negative correlation was observed between maternal parity with TNC and CD34+ cells though only the later was statistically significant. Ballen et al\(^{11}\) showed that primiparous female babies have a higher TNC and CD34+ cells which is probably due to prolonged labour
associated with first babies, which also must have reflected in this study. However Joseph et al and Nakagawa et al 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. 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. On the contrary, some other studies showed a significant positive correlation between birth weight and TNC and CD34+ cells. While Choong et al reported an association of 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. Thame et al 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 (>600g) have higher values of TNC and CD34+ cells while those with weight (<500g) have a lower values of cell count. A higher sample size in this study may have reflected the significance of the 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; however, it was not statistically significant. Jan et al.\textsuperscript{88} 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 \textit{et al.}\textsuperscript{92} found significantly more CD34+ cells in male babies. Some other studies showed that female sex was associated with a higher TNC count.\textsuperscript{74,85} The results in this study corroborated with the findings of M-Reboredo, who found no association between infant sex and cell counts.

There was no statistically significant correlation between cell count (TNC and CD34+cells) and mode of delivery though babies delivered through SVD had a higher TNC and CD34+ cells than those delivered by Caesarean section. A similar work by Aroviita shows no correlation between cell count and mode of delivery. In accordance with Sparrow, he observed that TNC was higher in cord blood collected from babies delivered by caesarean section\textsuperscript{93}. This difference in the reports of the mode of delivery could be due to a foetal response to the stress of labor.
CONCLUSION

This study showed that CD34+ cells, TNC and viability count of UCB at the University of Benin Teaching Hospital are within the acceptable value for Haematopoietic Stem cell transplantation.

Maternal parity was found to have an inverse relationship with TNC and CD34+cells yield from neonatal cord blood. There is a positive correlation between TNC and CD34+ cells reflecting the possibility of using TNC as an alternative to CD34+ estimation in centres that do not have facilities to estimate CD34+ cells. However, neonatal parameters did not significantly impact on the TNC and CD34+ cells yield.

LIMITATIONS

In this study the limitation include;

1) The study only evaluated cord blood of neonates within term and who had normal birth weight thus the impact of preterm and low birth weight on cord blood yield could not be conclusively evaluated.
RECOMMENDATIONS

Based on the observations made in this study, the following recommendations are made.

1) Samples should be processed within the shortest possible time in order to have a high viability count, TNC and CD34+ cells.

2) Cord blood volume should be assessed in subsequent studies, because it is one of the determinant factors parameters found to affect the final TNC and CD34+ cells in HSCT.

3) The sample size of 40 although above minimum accepted sample size if larger, could influence some of the parameters that had a difference but not statistically significant.
REFERENCES


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APPENDIX I

Ungated CD34+ cells
Gated CD34+ cells

Gated Viability Count
QUESTIONNAIRE

PLACENTAL UMBILICAL CORD BLOOD; DETERMINATION OF THE
SUITABILITY FOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION.

SECTION 1 (Mother)

Code Number;-

A. Demographic Data Of Mother

1. Age ……………….. Years

2. Ethnic group  (a) Bini  (b) Esan  (c) Urhobo  (d) Ibo  (e) Yoruba  (f) Hausa  (g) Others
   (specify)…………………

3. Genotype

4. Blood group

B. MEDICAL HISTORY

1. Hypertensive   (a) Yes  (b) No

2. Diabetic       (a) Yes  (b) No

3. Smoking       (a) Yes  (b) No

4. If Yes, no(s) of cigarette(s) per day ……………

5. Alcohol intake  (a) Yes  (b) No

6. Bleeding disorders   (a) Yes  (b) No

C. ANTENATAL PERIOD:-;

1. Last menstrual period

2. Parity.

3. Hypertensive   (a) Yes  (b) No

4. Gestational diabetic   (a) Yes  (b) No
SECTION 2

INVESTIGATIONS (Mother):

Hb/ Hct

INFECTION SCREENING

HIV

Syphilis

Hepatitis  HBsAg  HCV

URINALYSIS

SECTION 3 (Baby)

A. BIODATA OF BABY

Gestational age.-

Sex

Birth weight

Mode of delivery

Any physical abnormality

B. PLACENTA

Time of clamping;

Weight:

Time of collection of sample:  Time of processing:

SECTION 4 (Baby)

INVESTIGATIONS;

Total nucleated cell (TNC)

Viability testing of TNC

Total CD34+ count
INFORMED CONSENT

Patient’s Code Number:

TITLE OF STUDY: PLACENTA UMBILICAL CORD BLOOD: DETERMINATION OF THE SUITABILITY FOR HAEMATOPOIETIC STEM CELL TRANSPLANTATION AT THE UNIVERSITY OF BENIN TEACHING HOSPITAL, EDO STATE NIGERIA.

INVESTIGATOR: Dr. Ojo Matilda A O, Department of Haematology and Blood Transfusion, University of Benin Teaching Hospital (UBTH), Benin City, Edo state.

FINANCIAL SPONSORSHIP: This research is self-sponsored.

PURPOSE OF THE STUDY: Umbilical cord blood is often considered a biological waste and is usually discarded after delivery. However, it is a useful source of haematopoietic stem and progenitor cells for treatment of a wide variety of malignant and non-malignant disorders. Cord blood provides a readily available graft for patient, that do not have suitable matched related or unrelated donors. This study therefore aims to determine the suitability of cord blood for HSCT in Benin City Nigeria. As such, the outcome of this study will improve the practice of haematopoietic stem cell transplantation in Nigeria.

PROCEDURE INVOLVED IN THE STUDY: As a participant, you will be requested to volunteer answers to some questions in a questionnaire that will be given to you. In addition, you will voluntarily allow the collection of four (4) milliliters of blood from your baby’s placenta cord. You are being requested to participate in this study because these two criteria below required for this study apply to you and your baby.

1) Pregnant woman with uneventful antenatal period, also within the age of 18—40 years
2) Term baby with normal birth weight and no obvious congenital malformation.

**VOLUNTARY PARTICIPATION:** You are free to decide to participate in this study or not to participate in it. You are also free to withdraw from the study at any time if you so desire. Your refusal to participate or withdrawal from participation will not in any way affect your management/treatment.

**COMPENSATION:** There shall be no financial compensation for participating in this study.

**CONFIDENTIALITY:** The information provided in this study shall be treated with utmost confidentiality. The patient identity shall be concealed by using numbers instead of names. Information obtained will be stored in my personal computer and secured with a password. Other data will be in my file cupboard and locked securely.

**BENEFITS:** The study when completed will show the suitability of cord blood for haematopoietic stem cell transplantation for both malignant and non-malignant diseases. The study will also help to improve the practice of haematopoietic stem cell transplantation in Nigeria.

**RISKS:** There is no risk of collection of umbilical cord blood to both baby and mother, as the blood will be collected after delivery of the baby and the placenta.

Having read and understood the need for the study as well as the implications of participating in the study, I hereby freely give my consent. I have agreed to participate in this study.

Signature of participant………………………

Date ……………………………………

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ETHICS AND RESEARCH COMMITTEE
CLEARANCE CERTIFICATE

PROTOCOL NUMBER: ADM/E 22/A/VOL. VII/1084

PROJECT TITLE: "PLACENTA UMBILICAL CORD BLOOD: DETERMINATION OF THE SUITABILITY
FOR HAEAMATOPOIETIC STEM CELL TRANSPLANTATION AT THE UNIVERSITY OF
BENIN TEACHING HOSPITAL NIGERIA."

PRINCIPAL INVESTIGATOR(S)  DR. (MRS.) OJO MATILDA A.O.

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UNIVERSITY OF BENIN TEACHING HOSPITAL, BENIN CITY, NIGERIA

DATE CONSIDERED SEPTEMBER 1ST, 2014
DECISION OF THE COMMITTEE: APPROVED
REMARK:
CHAIRMAN: PROF. A.N. ONUNU

SUPERVISOR(S): DR. G.N. BAZUAYE, DR. E. ENABUDOSO
DECLARATION BY INVESTIGATOR(S):

PROTOCOL NUMBER (please quote in all enquiries)
To be completed in four and three copies returned to the secretary, Ethics and Research committee, Clinical services and
Training Division, University of Benin Teaching Hospital Benin City.

I/We fully understand the conditions under which I am/we are authorized to conduct the above mentioned research
and I/We undertake to resubmit the protocol to the Ethics and Research Committee.

Signature............................................................
Date............................................................