CORRELATION OF CD34+ STEM CELL YIELD DERIVED FROM UMBILICAL CORD BLOOD WITH BIRTH WEIGHT, GENDER, PLACENTAL WEIGHT AND GESTATION AGE AT KENYATTA NATIONAL HOSPITAL IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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JKUAT, Kenya.

DEDICATION

I dedicate this work to all the children suffering from leukemia at Kenyatta National Hospital. And all those patients suffering from Hematological neoplasia that can be treated by stem cell therapy in Kenya at large.

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I thank God for this far He has brought me. I wish to extend my sincere debt of gratitude to all those who contributed directly or indirectly in enabling me to successfully complete this work. First, I acknowledge my Supervisors: Dr. Kibet Shikuku and Dr. Michael Kahato for their tireless efforts in ensuring the completion of this work. I wish to thank KEMRI/Walter reed project my employer for giving me time to undertake my studies. Special thanks to Kenyatta National Hospital staff led by the labour ward staff for their support during collection of my cord blood samples and to all my research participants. Special thanks to KAVI flow cytometry section employees, Dr. Njoroge and Anne from BD for their help during gating of the cells in the flow cytometer.

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ABBREVIATIONS AND ACRONYMS

BFU	Burst-Forming Units
BPD	Biparietal Diameter
CB	Cord Blood
CFU	Colony-Forming Units
CPGH	Coast Provincial General Hospital
DC	Dendritic Cell
EDTA	Ethylene Diamine Tetra Acetic Acid
GVHD	Graft-Versus-Host-Disease
HLA	Human Leukocyte Antigen
HPC	Hematopoietic Progenitor Cell
HSC	Haematopoietic Stem Cell
KNH	Kenyatta National Hospital
MNC	Mononuclear Cell
NC	Nucleated Cell
PLTS	Platelets
RBC	Red Blood Cells

TNC	Total Nucleated Cells
UCB	Umbilical Cord Blood.
WBC	White Blood Cells

OPERATIONAL DEFINITIONS

CD34 Cell Yield- The number of CD34+ cells/ml of processed blood.

Haematopoietic Stem Cell (HSC)-Immature cell that give rise to other blood cells including white blood cells, red blood cells and platelets

Hematopoietic Progenitor Cell (HPC)-Cells that produce all blood lineages and arise from the hemogenic endothelium (HE) during embryogenesis.

Biparietal Diameter-It is a measurement of the diameter across your developing baby's skull, from one parietal bone to the other. The BPD is used to estimate foetal weight and gestational age.

ABSTRACT

Studies undertaken evaluating the correlation between CD34+ cell yields in umbilical cord blood against birth weight, gestation age, placental weight and gender have shown different findings across different populations. Since no study has been done in our set up, this study aimed at characterizing factors that affect CD34+ cell yields in umbilical cord blood (UCB) samples in Kenyan women seen at KNH. Some of the physiological factors of interest in the study included: gender, placental weight, infant birth weight, and gestation stage. The specific research study objectives were: To determine the CD34+ cell yield/ml of umbilical cord blood, to determine the correlation between CD34+ cell yield from UCB to Gestation age, birth weight, Placental weight and Gender of the baby and to compare CD34+cell yield between normal deliveries and caesarean section (CS) deliveries. The study null hypothesis stated that there is no correlation between CD34 +cell yield with gestation age, birth weight, placental weight and gender. This was a prospective hospital-based cross-sectional study enrolling consenting expectant mothers seen at Kenyatta National Hospital (KNH) where consecutive sampling was used. A total of 79 mothers meeting the enrolment criteria had samples taken by a trained nurse in charge of normal deliveries by clamping the umbilical cord. Mothers going for CS had samples collected in theatre using the same technique. Acquired samples were batched and analysed using a FACSCalibre machine for CD34+ testing. Using a questionnaire, the gestational age was obtained, while the birth, placental weight and gender of the baby were obtained using a data collection sheet. A Microsoft excel spreadsheet was used to collect all data and analysis was done in SPSS version 20 software. The findings showed that youngest client was 19 years and the oldest was 33 years with a mean age of 24 years. Qualitative variables were described as absolute and relative frequencies, while quantitative variables were described as means ± standard deviations. The Chi-squared test ($\chi 2$) test was used to test the homogeneity of proportions. Prevalence ratios (PR) and 95% confidence intervals (95% CI) were calculated. The level of significance is set for a p-value <0.05. Ethical clearance was obtained from KNH/UON-ERC. A p value of ($p=0.00$) was obtained which is lesser than 0.05 It showed that there is a high positive relationship between CD+34 cells and gestation age, birth weight, placental weight and gender. This study concludes that the higher the birth weight, placental weight, vaginal means of giving birth the higher the number of CD34+ cells. The longer the gestations age the more the CD34+cells and hence these should be the preferred samples for banking or treatment of the various diseases.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The clinical applications of umbilical cord blood (UCB) has grown since the first haematopoietic stem cell (HSC) transplant for a patient with Fanconi anaemia in 1988 (Gluckman *et.al.,*2000). There is continued improvement in HSC transplantation using UCB as a graft source. Multi-institutional trials of UCB transplant are going on and novel methods of cell processing are increasingly incorporated into investigational trials involving UCB-derived haematopoietic and non-HSCs as well as immune cells. Worldwide, more than 220,000 umbilical cord blood (UCB) units have been Human leukocyte antigen HLA-typed and kept in banks and more than 6000 UCB transplants have been performed in children and adults (Hassall *et al.,* 2003).

Cord blood is rich in haematopoietic (blood forming) stem cells that are used to treat over 80 blood related diseases. Cord blood is a valuable source of stem cells for a bone marrow transplant and can be used to replace diseased cells with healthy new cells, and rebuild an individual's blood and immune system. (Eaves *et.al*., 2015)

Cord blood is collected immediately after the birth of your baby by your obstetrician or midwife. The umbilical cord is cut and clamped, a needle is inserted into the umbilical vein and blood is collected into a sterile collection bag. Cord blood collection is quick, safe and painless for both mother and baby.Approximately 100-150ml of blood needs to be collected for successful storage. (Ballen *et.al*.,2008)

In our bodies, bone marrow is the source of all blood cells.Haematopoietic stem cells are contained in the bone marrow and they continuously make new blood cells to replace old ones.If bone marrow is damaged by disease or medication, it cannot make these essential blood cells, leading to fatal consequences. Therefore, haematopoietic stem cells

must be replaced as part of the treatment.This is done via whole bone marrow transplant or stem cell transplant.Cord blood is an alternative source of stem cell for a transplant. (Smith *et.al*.,2009)

The first UCB bank was established by Brookmeyer. This bank provided the unit for the aforementioned first transplant as well as for human leukocyte antigen (HLA)-matched sibling transplant. As UCB is continuing to gain acceptance as an alternative to marrow and peripheral blood in the related transplant setting, the potential for the establishment of unrelated UCB banks became apparent. Since that time, banks have grown substantially with a reported 450,000 unrelated units currently banked worldwide according to world marrow donor association in 2005 (Broxymeyer, 2005).

This therapy known as stem cell transplantation has traditionally been performed using bone marrow or peripheral blood as source of hematopoietic stem cells but in many circumstances stem cells in umbilical cord blood has given a lower risk of graft vs. host disease, HLA-mismatch tolerance, lower costs, non-existent risk to the donor, more expeditious time to obtainment and less infectious morbidity (Barker *et al.,* 2010).

According to Rocha, CD 34+ stem cells are pluripotent hematopoietic stem cells that give rise to all cells in the blood including red cells (RBCs), platelets (PLTS), Neutrophils, Basophils, monocytes and lymphocytes (Gluckman *et al* 2000). Several studies from Europe (Jones *et al*., 2003), Japan (Nakagawa *et al.,* 2004), Taiwan (Jan *et al*., 2008) and the United States (Ballen *et al.* 2001) have examined the various factors that improve the quality of the collected UCB units. Some of the variables that were identified included maternal factors such as mother's age, race, number of previous births, smoking status and foetal factors such as weight, sex, placental weight and umbilical cord length. The rationale being that it would be useful to predict UCB cell content using information of donor-related variables before collection and cell processing (Aroviita *et al.,* 2005). Efficacy of cord blood transplantation correlates in large part with total nucleated cell or progenitor cell (CD34-positive cells). Faster

engraftment and better survival are achieved, for example, when cord blood grafts provide at least 2.0×10^7 TNC per kilogram of body weight (Migliaccio *et al.*, 2000).

The volume of cord blood collected along with its nucleated cell concentration determines the total number of nucleated cells that are available in individual cord blood units. Hence obtaining an adequate volume of blood affects the suitability of the unit for transplantation and patient survival (Majhail *et al.,* 2006).

The ability of the foetus to grow and thrive in utero depends on the placental function and the average weight of the placenta at term is 508 g. The ratio between placenta weight and birth weight of the new-born is 1:6. However, methods of measurement vary widely particularly due to differences in placental preparations. Placental weight and its relationship to infant size at birth have been studied for more than a century. Past studies indicated that placental weight was associated with pregnancy outcome. High placenta weight was associated with a poor perinatal outcome, a low Apgar score, respiratory distress syndrome and perinatal death; whereas a low placental weight was associated with medical complications in the mother. Barker *et al* 2010,

Reported that altered growth of the placenta was a predictor of maternal medical diseases including cardiovascular disease, hypertension and diabetes mellitus. Other factors such as race and socioeconomic status also affect the placental weight. The main limitation factor for wide use of umbilical cord blood units (UCBs) as a source of hematopoietic progenitors for transplantation is cell dose. International standard guidelines recommend 2 x 10(7)/kg as the minimal nucleated cell dose for UCB transplantation for adults and 3.7 x 10(7)/kg for children. Therefore, it is important to the optimize donor selection and the collection method so as to achieve high cell doses.

Stem Cells are the cells that make up the embryo and are the original building blocks of life. Stem cells develop into various cell types in the body such as: skin, blood cells, muscle, bone, nerves and cartilage. (Steel *et al.*, 2016).

After birth, stem cells are found all over our bodies and they serve to repair and maintain our body cells throughout our lives. Stem cells are abundant in the umbilical cord blood and tissue and can easily be collected at the birth of the baby (less invasive than harvesting them later in life). They are normally discarded as medical waste, making their collection free of moral, ethical and religious concerns. (Servais *et.al*.,2015).

The aim of this study therefore is to identify obstetric factors that influence the CD34+cell concentration and especially those factors that identify deliveries that the obstetrician might be able to modify to increase the yield.

1.2 Problem Statement

Umbilical cord blood banks have been established in countries throughout Europe, Asia, North and South America and Australia. There is currently no public cord blood bank in Africa (Crookes *et al*., 2007) including Kenya. Umbilical cord blood (UCB) has become an alternative source for providing hematopoietic stem/progenitor cells as well as non-hematopoietic stem cells, compared to the conventional sources of bone marrow (BM) and peripheral blood (PB). Thus, this study aimed at characterizing components that comprise UCB samples and the physiological factors that affect them which include: gender, placental weight, Infant birth weight and gestation stage. The study was to help determine levels of CD34+ cells and selection criteria for cord blood donors since it is difficult to predict the optimal cord blood units in advance because of extremely wide variations in individual samples.

1.3 Justification

This study was being done as part of creating knowledge pool for the future. From this information, it can be decided whether a sample has the minimum number of CD34+ stem cells in relation to gender, placental weight, gestation age and birth weight.

This knowledge will facilitate the selection of optimal cord blood samples for unrelated banking and the early discarding of sub optimal cord blood samples thus resulting in the saving of costs related to expensive further processing.

1.4 Research Questions

1. What is the CD34+ cell yield/ml of umbilical cord blood?
2. What is the correlation of CD34+ yielded from Umbilical cord blood to Gestation age, Birth weight, Placental weight and Gender?
3. Is there a comparison of the CD34+cell yield between normal deliveries and caesarean deliveries?

1.5 Hypothesis

1.5.1 Null Hypothesis

There is no correlation between CD34 +cell yield with gestation age, birth weight, placental weight and gender.

1.5.2 Alternate Hypothesis

There is correlation between CD34 +cell yield with gestation age, birth weight, placental weight and gender.

1.6 Objectives

1.6.1 General Objective

To determine the correlation between CD34+ cell yield from umbilical cord blood and gestation age, birth weight, placental weight and gender.

1.6.2 Specific Objectives

1. To evaluate CD34+ cell yield/ul of cord blood.
2. To determine the correlation between CD34+cell yield to gestation age, birth weight, placental weight and gender.
3. To compare the CD34+cell yield between normal deliveries and caesarean deliveries.

CHAPTER TWO

LITERATURE REVIEW

2.1 Umbilical cord blood as a target for hematopoietic stem cell expansion.

Umbilical cord blood (UCB) is a target for Hematopoietic stem cell expansion, as collection of a particular unit is a one-time event and the volume is limited relative to marrow and peripheral blood. It is shown to have greater self-renewal capacity when compared to other sources of HSCs hence one would expect HSC expansion using UCB to have a greater probability of success (Servais *et.al*.,2015).

Before the usefulness of mbilical cord blood in medicine was found, the umbilical cord was treated as medical waste and was disposed.Today we can store it long term without losing its greatest value which are the stem cells. Collection of umbilical cord blood is safe and painless. The procedure is non-invasive and short . It takes place after child is born and does not threaten mothers or child's life or health. The blood may be collected during natural spontenous labours as well as cesarean section.(Ballen *et al.,*2008).

The White blood cells population in cord blood is primarily composed of the mature granulocytes that generally account for approximately 55% to 65% of the total nucleated cell cell content. The remaining mononuclear cell (MNC) population in CB (35%-45% of TNC) includes lymphocytes, monocytes, and CD34+ and CD34- hematopoietic stem/progenitor cell (HPC). Compared to mature granulocytes, MNCs are capable of engrafting in cord blood transplant recipients and thus make a contribution in transplant outcomes (Korbling & Anderlini 2001). The first reports of related and unrelated donor UCB transplantation were the proof of the concept that UCB can be used as a source of HSC transplantation (Ballen *et al.,* 2001). It is noted the field of UCB transplantation has advanced much faster in paediatrics compared to adults as the ability to find suitable UCB is greater for younger patients (Barker *et al.,* 2001). In recent years, there have

been more reports on the use of cord blood for the treatment of metabolic diseases, immune deficiencies and haemoglobinopathies. (Cohen & Nagler, 2007).

Cord blood can be obtained at birth, taking advantage of the existence of cord blood banks that collect cord blood from donors. Other studies suggest that the use of cord blood induces less adverse symptoms due to the presence of naive regulatory T cells in the cord blood. CD34+cells in the marrow give rise to circulating DC precursors that is home to tissues, where they reside as immature cells and then they mature upon antigen encounter (Gluckman *et al*., 2000).

Stem cells are undifferentiated and unspecialized systemic cells characterized by self-renewal by successive divisions. These cells can originate from a clone of which their descendants can differentiate and give rise to specialized cells enabling the potential to repair their damaged tissue or replace it. Surface molecules are utilized to identify these stem cells. One important marker is the CD34 antigent hat is expressed on most stem cells (steel *et.al* 2016).

Banking of the umbilical cord blood is the process by which collection and storage of umbilical cord blood is carried out immediately after birth. Some of the cord blood banks (CBBs) provide not only cord blood banking but also banking of umbilical cord tissues, where a small segment of the umbilical cord itself is cut, preserved and stored.The interest in umbilical cord tissues is growing,since these tissues are considered as a very rich source of mesenchymal stem cells, which provide a great potential for the field of the regenerative medicine. Many clinical trials are currently investigating the use of the Cord-derived hematopoietic as well as mesenchymal stem cells for the neurological and autoimmune disorders such as autism, cerebral palsy and type 1 diabetes.

Placental cord blood has been shown to have sufficient progenitor cells to provide durable engraftment.The first related cord blood transplant was performed in 1988.The

largest single institution experience is at Duke University,(Rubinstein *et al.,* 2003) that have recently reported on the results of 562 cord blood transplants that were facilitated by the New York Blood Center. In this study, engraftment was dependent upon the nucleated cell count of the cord blood units. In the Eurocord study of the 60 unrelated cord blood transplants the nucleated cell dose/kg infused correlated with engraftment of the cells..Many studies have been reported regarding these obstetric and neonatal factors that can affect quality and quantity of CD34+ stem cells.There are many potential advantages in using cord blood stem cells as opposed to adult bone marrow-derived hematopoietic stem cells (HSC).

UCB stem cells appear to be more potent than HSC's extracted from bone marrow. It has been demonstrated that 80-120 ml of UCB contains as many hematopoietic stem cells as 1200 ml of the bone marrow aspirate. The quality of the UCB stem cell graft is superiorcompared to that of HSC derived from bone marrow, although cord blood contains less mesenchymal stem cells. In the Unites States, there are 4.5 million deliveries every year. In the vast majority of these cases, the umbilical cord blood is discarded with their placenta.Cord blood stem cells are readily available and easy to process. Extraction of stem cells from cord blood is a simpler process and the time from collection to transplant is shorter than HSC extraction from the adult donors (salter *et.al*.,2009).

The graft rejection risk is lower for UCB stem cells as opposed to HSC extracted from adult donors. These cord blood stem cells are not antigenically mature. Full HLA compatibility before transplant of UCB is not required,hence allows transplantation from a wider donor pool. Because cord blood stem cells are immunologically immature, the fetal immune system rarely creates antibodies. The success rates of grafts using UCB stem cells are quite similar to those in bone marrow but higher than those of peripheral blood. (Rosenau *et al*.,2012).

The cell replication from UCB stem cells is superior to that of HSC's derived from adult bone marrow presumably due to their longer telomere length. Cord blood also contains their mesenchymal stem cells which are non-hematopoietic progenitor cells. These mesenchymal stems cells can differentiate into many types of mature cells:such as neuronal cells, osteoblasts, adipocytes and chondroblasts. As opposed to bone marrow aspiration from an adult donor, there is little or no associated risks of mortality from collecting umbilical cord blood (Broxymerey *et al.*,2005). Cytomegalovirus infection risk using UCB is lower than that in adult bone marrow, which reduces the post-transplant complications.On the other side UCB stem cells are more primitive compared to peripheral blood and bone marrow stem cells; therefore, immune system recovery time takes longer. This may put the patient at risk of infection for a longer period of recovery time.The volume of collected UCB stem cells is smaller compared to that recovered from the bone marrow aspirate of a an adult. Acording to Gwendolin *et al.*,2008,This is an important limitative factor for a successful graft. Also, the viability of UCB stem cells after cryopreservation is still very undetermined. A10-year period storage of the umbilical cord blood stem cells is the current gold standard. Longer storage times may be feasible yes though this has not been studied yet Hence CD34+ and CD90+ cell content are useful for the evaluation of the quality of cord blood units for future hematological and non-hematological therapeutics.

CD90 (Thy-1), a small GPI-anchored protein, plays a role in cell to cell interaction events, including intracellula cell recognition during development. It is considered as an important stem cell marker to identify the mesenchymal stem cells (MSCs) in vitro . Further, it has been observed to be expressed in other kinds of the stem cells such as hematopoietic progenitor cells and hepatic progenitor cells in the human fetal liver. HSCs (CD34+) accomplished reconstitution of the human hematopoiesis in severe combined the immune-deficient (SCID) mice that expresses Thy-1 antigen. The Thy-1 is involved in hematopoietic cell development,by mediating a negative signal that may result in inhibition of cell proliferation. Thy-1 is expressed the both primitive HSCs and

non primitive MSCs.Transplantations using umbilical cord blood are still in their research phase and variables that might improve the quality of blood are currently the focus of these research, in as much as knowing these factors may result in their lower costs and less waste of time in the processing and storage of the material (Smith *et.al*.,2009).

One of the limitations of this type of transplant is their volume and their contents of the blood collected from the umbilical cord which can be an obstacle to the hematopoietic stem cell grafting. The main parameters used in cord blood banks include the total nucleated cell count, percentage of CD34+ cells and the volume of the blood. (Nakagawa *et.al*., 2004).

The Umbilical cord blood (UCB) from the related and the unrelated donors, is broadly an alternate donor source for allogeneic transplantation in children as well as adults for the treatment of the various hematological and non-hematological malignant disorders. The major challenge associated with cord blood is their cell content. A strong correlation of the number of total CD34+ (classical hematopoietic stem cell marker) cells with rates of engraftment following UCB transplants have been documented by some of these studies. An association of hematopoietic stem cells (HSCs) CD34+ content of cord blood with their maternal and neonatal parameters has been previously observed. (Rosenau *et.al*.,2012).

2.2 Obstetric factors that influence CD34 cell yield in Cord blood and their Influence on the Cell types.

Umbilical Cord Blood (UCB) and the umbilical cord itself have been reported to contain other stem/progenitor cell types, including mesenchymal stem/stromal cells and endothelial progenitor cells. These overall types may have the potential to improve transplant outcomes when used in conjunction with HSC cell to bone marrow and peripheral blood for HSC transplantation in both children and adults (Salter *et al*., 2009).

It has the advantage that it can be tissue typed, screened for viral biomarkers, processed and banked, especially ahead of urgent or directed transplants, without the attrition rate that occurs with bone marrow donors. Since the first UCB HSC transplants in 1988 a great deal of knowledge has been acquired on the optimal selection of UCB units for HSC transplantation (Barker *et al.,* 2010). This is based on obstetric factors, total nucleated cell content (TNC), CD34cell count, HLA and blood group matching of the recipient and donor plus processing, storage and Typically, UCB is collected from a wide range of donors, held for a period of time prior to processing, and then cryo-preserved under controlled rate freezing conditions and stored long-term below-150°C (as recommended by international accreditation agencies such as FACT-Net cord) (Watt *et al.,* 2010).

Units are also analysed before or after processing for TNC, CD34+cell numbers and hematopoietic colony forming units (Avery *et al.,* 2010). Obstetric factors that have previously been reported to affect TNC and CD34 cell content include birth and placental weight, infant gender, parity of the mother, nucleated red cell content, low venous ph., prolonged first stage of labour and Apgar scores quantifies the ability of the infant to adapt to the extra uterine environment and hence a measurement of the health of new born infants at 1, 5 and 10 min post birth (Apgar 1m, Apgar 5min, Apgar 10min) with the normal Apgar score varying from 7 to 10.

The New York Blood Centre harvests the cord blood from the delivered placenta and has reported an association between volume of collection and the umbilical cord length. Nucleated cell counts were similar among different racial groups but $CD34^+$ cell counts were lower for blacks in the Midwest and North Carolina collection sites. (crookes *et al.*,2007). Biometric parameters (BPD, abdominal circumference, femoral length and head circumference, evaluated through ultrasound at the end of the gestational period correlated with WBC count and CD34+ cell count. In particular, WBC count correlated positively with head circumference and BPD but not with abdominal circumference or femoral length. $CD34^+$ cell count showed a statistically significant correlation with

abdominal circumference, but not with head circumference, BPD or femoral length (Urciuoli *et al.,* 2010) According to literature, T-cells are involved in cell-mediated immunity, there are many subsets of T-cells and those that have been examined include activated T-cells, those that have been stimulated by antigens and have the function of T-helper, T-cytotoxic cells and regulatory T-cells, those that are involved in immunological tolerance by stopping T-cell mediated immunity at the end of an immune response and suppressing auto-immune reactions (Yang *et al.*,2011). The most interesting effect on T-cells has been shown by obstetric history; increased gravidity is inversely proportional to T-cell concentration. Again, this could have an impact on the immunity of infants born late into larger families. Mother's age also negatively impacts T-cell concentration and indeed all UCB lymphocytes with older mothers producing offspring with lower T-cell concentration. However longer gestation and higher birth weights equate to higher T-cell concentrations supporting the view that babies who are born at full term are at lower risk than those born prematurely (Tommiska *et al.,* 2001). According to Li in 2006 another UCB cellular sub-type that has been examined is B-cells (Urciuoli *et al.,* 2010). Dendritic cells (DC) are professional antigen presenting cells and are of paramount importance in the immune response. Both the birth weight and obstetric history have high impact on the levels of DC concentration found in the UCB. The fact that a lower birth weight means lower DC concentrations is of a great concern. Premature babies therefore are likely to have more problems with immunity compared to their full-term counterparts. Again, those babies born to mothers with more previous children are likely to suffer this same fate (Cools *et al*.,2007).

To establish a CBB, the number of CD34+ cells in each cord blood unit (CBU) must be adequate, that’s why obstetric and neonatal factors should be considered when selecting these CBUs.Many factors have been suggested to influence the quantity and quality of CD34+ Cell derived from the cord blood, and that may be responsible for these variations in the reported results. Among these factors; the birth weight, sex of the new

born and maternal age may have an effect on the concentration of the CD34+ cells. (Steel *et.al* 2016).

In medical practice, there has been a dramatic increase in the use of these umbilical cord blood (UCB) Stem cells for the treatment of hematopoietic malignancies and blood diseases. Current research is ongoing to determine the feasibility of UCB stem cells for use in drug sensitivity investigations, therapies and regenerative medicine (Steinborn *et.al* 1999).

The majority of the stored UCB in the United States is for the private use of their families, in the event of malignancy diagnosis in the child or a relative. At present, the cost of sample preparation and storage has limited widespread acceptance of UCB as a public health resource. It was reported that more than half of the collected samples in some of UCB banks are discarded, either due to their low volume of total nucleated cells (TNC) or due to obstetric factors. (Solves, P. *et al*., 2004.)

In previous studies (Omori *et al.*, 2010, Sparrow *et al*., 2002) UCB TNC affects CD34+ cells count positively. There is a great hidden promise in this significant correlation as many efforts are focused on cell expansion and CD34+ cells proliferation that can be able increase the usefulness of UCB during transplantation. These correlation might help in conducting more studies on the cord blood cellular content.

According to Servais *et al*., (2015), it is essential to know the donor obstetric profile and which factors might influence the quality and quantity of the product. Higher TNC count is thought to achieve better results in the hematopoietic stem cell transplantation. Many obstetrical factors can influence the choice of using a single UCB stem cell donor for their transplant including blood group typing, HLA typing, neonatal gender, parity, low venous pH, prolonged first stage of labor and Apgar scores. Technical factors can also influence the choice of a single UCB stem cell donation such as TNC, CD34+ count, procedures for processing and storage time.

Recent studies Yang *et al*., (2011) and Hrushesky *et al*., (2011) report that some variables affect the quality of the umbilical cord blood, especially those related to obstetric and foetal features such as placental weight, birth weight, gestational age (GA), route of delivery, gender of the newborn, among other factors. thus research is being developed in these area to attempt to improve cell levels, which is essential to increasing the grafting success rates.

2.3 Method of delivery and their influence on CD34 cell yields

The utilization of UCB has widely become an easily available and acceptable alternative for the stem cell transplantation of non haematological as well as haematological disorders. In the last decades, the potential of non-oncologic stem cell and mononuclear cell therapies have been investigated for the regeneration of the impaired organs and tissue regeneration (Kogler, *et.al* 2009). In clinical settings, the infusion of UCB in infants with these neurological disorders seems feasible and safe. (Allison J *et.al* 2010). That's why there is need to select the optimum units to be used. It has been suggested that spontaneous labours are accompanied with more CD 34+ cell counts. (Sparrow *et al.,* 2002). Labour in vaginal delivery is a very stressful experience that has been associated with more levels of various granulolyctes, including monocytes (Glasser *et.al* 2015) and lymphocytes (Gluckman *et.al* 2000). During labour, the activation of cytokines has been found in the systemic maternal circulation (Watt *et.al* 2010).It is assumed that the increased metabolic activity which may result from the intensified activity of uterus muscles and skeletal muscles as well as accompanying oxygen consumption may be able to lead to increased production of reactive forms of oxygen (intensified oxidative stress) in the course of these spontenous labours. In this scenario, the organism responds with an increased stem cell production in the blood of the fetus, these assumptions were confirmed by Aufderhaar *et al.*2003. Eaves *et al*.,2015, confirms that low pH correlates with the number of MNCs, and hence the number of CFU-GM increases proportionally to the length of the first stage of labor. Aufderhaar et.al 2003, showed that these perinatal factors, such as prolonged 1st stage of labor and low blood

pH correlate with the increase of the number of nucleated cells, CD34+ cells, granulocytes, and progenitor cells in the umbilical cord blood. However, there is a group of scientists who have observed in their studies a more concentration of nucleated cells in the cord blood collected in the course of C-sections (Servais *et al.*,2015). Ballen *et al.*2001, reported a more activity of neutrophils and their concentration after C-sections. Hence the need to find out which method to use in our setting to be able to collect optimum units of cord blood.

The morbidity and mortality associated with allogenic bone marrow transplantation is still more so it is necessary to find alternative sources of stem cells. Among those alternative sources are umbilical cord blood and placental blood when evaluated as very useful. Umbilical cord blood has now been established as the main source of stem cell transplantion for patients suffering from these various diseases (Jaime-Pérez, J. C. *et al.*, 2011).Current data: Manegold, G. *et al*.,2008 and Servais *et.al*.,2015 suggests that the umbilical cord blood is the most acceptable source of transplantable hematopoietic stem cells and non haemotopoietic cells at least in recipients <40kg. As a response to these clinical results of cord blood transplants, several cord blood banks have been established worldwide. Programs have been funded by both private and public organizations but the limited resources available force the cord blood banks available to limit the number of umbilical cord blood units to process and freeze.

Based on these available literature, there are significant discrepancies among recommendations for improving the quality of UCB stem cell samples. These differences arise from the wide range of populations represented in the published studies. Therefore, precise, population-based information is required to be able to determine optimum predictors for high yield TNC/CD34+ cells for UCB stem cell sampling.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted at Kenyatta National Hospital in Kenya. It is a National Referral and Teaching Hospital and provides medical research environment. It is currently the largest referral and teaching hospital in the country with approximately 30 to 50 births per day.

3.2 Study design

This study was a prospective hospital-based cross-sectional study design. Samples were obtained from cords delivered in labour ward at KNH. Consecutive sampling was used to enrol consenting expectant mothers.

3.3 Study Population

Expectant mothers who were in the labour ward at KNH. Written informed consent was obtained from each participant before obtaining any information. It involved an ongoing conversation between the researcher and the participants before, during and after the research.

3.3.1 Inclusion Criteria

- All expectant mothers who consent and have dully filled the forms.
- All expectant mothers who deliver within the third trimester either through normal delivery or caesarean section

3.3.2 Exclusion Criteria

- All expectant mothers who get still births
- All deliveries not within the third trimester.
- All monozygotic twins.
- All expectant mothers with infection of the foetus.

3.4 Sample Size Determination

Sample size determination was done using Prasanna (2013) Sample size determination formulae;

$$\text{SS} = \frac{Z^2 * (p) * (1-p)}{C^2}$$

Where: Z = 1.96 for 95% confidence level

p = percentage incidence (0.5)

q = (1 – 0.5)

C = confidence interval, expressed as decimal, (0.05).

Since the population of the mothers giving birth in a day are known the formulae is subjected to a correction factor for a finite population;

$$\text{New SS} = \frac{SS}{1+\frac{SS-1}{pop}} = 37.81$$ Where: pop = population visiting the maternity facility

Therefore 37.81 + 100 gives a total of New SS $= \frac{SS}{1+\frac{SS-1}{pop}} = 35.61$

The total therefore is 37.81 + 35.61 = approximately 73 mothers.

n= 73

To be able to attain a sample size of 73, a total of 146 mothers were interviewed.

3.5 Sampling Method

The study used purposive sampling techniques to consecutively enrol every consenting mother who meets the inclusion criteria until the minimum required sample size was obtained. A total of 73 mothers were enrolled to the study.

3.6 Data Collection

Consenting participants provided information based on a structured data collection sheet. The nurse took detailed information on patient as per the sheet.

3.7 Laboratory Procedures

Sample collection was done after clamping of the cord by medical personnel (a nurse in charge of the delivery after a short training session). A hook was attached to a metal rod like a retort stand and the clamped umbilical cord passed through a hole cut. The hook was raised up and clamped higher up the stand such that the cord hung down with its full length suspended and the umbilical cord vein filled with blood by gravity. The entire cord was disinfected twice with 70% isopropyl alcohol and the intended venepuncture site which is the distal end of the cord just above where it was clamped, swabbed with 2% povidone iodine tincture, aseptic and vacuum system tubes and collection kits were used. 4ml of Anti-coagulated blood was obtained. The anticoagulant of choice was ethylene diamine tetra acetic acid (EDTA). Specimens were collected within the first five minutes after clamping of the cord and labelled according to the corresponding

number of the sheet of the expectant mother. The samples were then transported in a cooler box to the laboratory and stored at 4^{o}C if necessary, for less than 24 hours.

Universal precautions and attention to asepsis were observed throughout the procedure and a gynaecologist was overlooking the whole procedure.

3.7.1 Laboratory Assay

Laboratory assay was done with FACSCalibre machine using (CD45 FITC+/CD34 PE). 2.5 ml of blood was used for CD34+ testing Samples were mixed in a rocker. Sample IDs were added into the work list on the FACS Calibre computer. Two Trucount tubes were arranged on a rack alongside the samples on the work bench and labelled. The labelled tubes were transferred to the hood and 10 micro litres of mAb added to the appropriate tubes. The specimen was inverted gently 15-10 times to mix and the stopper removed carefully. Fifty microlitres of blood was added to each tube by reverse pipetting. The Trucount tube were recapped and vortexed gently to mix. The tubes were wrapped with aluminium foil and incubated at room temperature (RT) (18-25^{0}C) in the dark for 15 minutes. The tubes were returned to the hood and 450 micro litres of diluted FACSLyse added to each tube and vortexed. The tubes were wrapped with aluminium and incubated in room temperature until ready to lyse the red blood cells. The samples were analysed in the FACSCalibur machine. All variables and other details were collected on the form and CD34+ cell yield was reported per ml of blood.

3.7.2 Quality Assurance

Standard operating procedures were used and manufacturer's instructions followed as indicated in Appendix III. Two extra tubes were included for normal and low-quality controls (QC). Participants' identification numbers on the sample was matched with the request forms to avoid errors.

3.8 Data Management

All collected data was cleaned for any errors and kept secure through use of encryption to protect unauthorised personnel from accessing the files. Confidentiality was censured by use of passwords. The nurse assistants received appropriate training on securing and maintaining confidentiality and safeguarding data. Data was coded in Statistical Package for Social Sciences SPSS®(2016) version 20.0 software for analysis and archiving any other identifiable confidential data were destroyed.

3.9 Statistical Analysis

Collected data were stored in Microsoft excel and later exported to the statistical package for social sciences SPSS ® (2016) (version 20.0) for analysis and correlation. Qualitative variables were described as absolute and relative frequencies, while quantitative variables were described as means ± standard deviations. The Chi-squared test ($\chi 2$) tests were used to test the homogeneity of proportions. Prevalence ratios (PR) and 95% confidence intervals (95% CI) were calculated. The level of significance was set for a p-value <0.05.

3.10 Ethical Considerations

Ethical clearance was obtained from KNH/UON Ethical Review Committee. (KNH-ERC/A/56)

CHAPTER FOUR

RESULTS

Findings are presented based on the objectives that the study sought to investigate the correlation between CD34+ stem cells yield derived from umbilical cord blood with

birth weight, gender, placental weight and gestation age at Kenyatta National Hospital in Kenya.

4.1 Characteristics of the study Population.

The study participants were women within their mid reproductive age group with a mean age of 25 and a gestation of 36 weeks. Most of the babies weighed at least 3100 grams and the placental weight average was 510.05 grams with a median of 525.5 grams (Table 4.1.)

Table 4.1: Characteristics of the study Population.

Variable	Sample Size (N)	Mean	Median	Standard Deviation
Mothers Age	73	24.46	25.00	1.268
Gestation Age	73	35.87	39.00	0.463
Birth Weight	73	3120.47	3150.00	2.433
Placental Weight	73	510.05	525.500	1.186

4.2 Respondents Demographic Background

4.2.1 Previous Births

The researcher sought to find out the number of births that the women had had in the past. The respondents gave their responses which are reflected in table 4.2. below.

Table 4.2: Previous Births

Number of Previous Births	Frequency	Percent
One	22	30.4
Two	12	19.0
Three	4	5.1
Four	3	3.8
None	32	41.8
Total	**73**	**100.0**

The finding reflects that thirty-two (32) respondents had not given birth prior to the current birth during the study; they represented 41.8% of the sampled population. 30.4% of the sampled population did record to have had one birth previously with a representation of twenty-two (22) respondents. Twelve (12) respondents did record that they have had two previous births with a representation of 19.0% of the sampled population. Four (4) respondents did record that they have had three (3) births in the past prior to the current one, they represented 5.1% of the sampled population. Only three (3) respondents did record to have had four (4) births in the past with a presentation of 3.8% of the sampled population.

4.3 CD34+ cell yield/ml of cord blood.

CD34+ cells were analysed using flow cytometry and the yields clustered into 6 groups according to percentage gating, namely: less than 1%, 1% - 1.5%, 1.6% - 2.0%, 2.1% - 2.5%, 2.6% - 3.0%. 55.7% (41) of the participants had a CD34+ cell yield/ml in the cluster group of 1.6% - 2.0%, followed closely by 17.7% (13) with a cell yield of 2.1% - 2.5%. Other cluster groups were: 1% - 1.5% with 11 participants (16.5%), 2.6%-3.0% with 6 participants (7.6%), less than 1% with 2 participants (2.5%).

Table 4.3: Shows CD34+ cell yield/ml of cord blood using flow Cytometry and corresponding percentages

CD34+ Cell Yield/ml	Frequency	Percent
Less than 1%	2	2.5
1% - 1.5%	11	16.5
1.6% - 2.0%	41	55.7
2.1% -2.5%	13	17.7
2.6% - 3.0%	6	7.6
Total	**73**	**100.0**

4.4 Correlation between CD34+cell yield to gestation age, birth weight, placental weight and gender.

The correlation between CD34+ cell yield and gestation age, birth weight, placental weight and gender was determined using Pearson correlation. The reported p-values for associations (as well as the total and partial R2) between CD34+ cells and birth weight, placental weight and gender variables from generalized linear mixed models. The results pinpointed the relevance of a covariate for the generalized linear model and clearly showed that gender was the only variable with not statistically significant covariates associated to CD34+ cells with outcomes ($p > 0.857$) which is greater than 0.05. This was done at 20 degrees of freedom. As for Placental Weight, and Birth Weight, significant covariates were: ($p < 0.000$), for all of the variables other than gender. The findings reflect that the data processed through correlation analysis to find the relationship between CD34+ Cells and Gestation Age showed there was low positive relationship between CD34+ Cells and Gestation Age. This was done at 95% (0.5) confidence level and a significant relationship of $p = 0.04$ and $r = 0.000$. This depicted

an indication that CD34+ cell has a positive relationship with birth weight, placental weight and gestation age. There was no relationship between gender and CD34+cell yield. The findings are reflected on table 4.4. Below:

Table 4.4: correlation table showing the relationship between CD34+ and Birth Weight, Placental Weight and Gender

Correlations						
		CD34 +	**Birth Weight**	**Gestation Age**	**Placental Weight**	**Gender**
CD34 +	Pearson Correlation	1	.000	.004	.000	.857
	Sig. (2-tailed)		.000	0.00	.000	.853
	N	73	73	73	73	73
Birth Weight	Pearson Correlation	.000	1			
	Sig. (2-tailed)	.000				
	N	73	73	73	73	73
Gestation Age	Pearson Correlation	.004		1		
	Sig. (2-tailed)	0.00				
	N	73	73	73	73	73
Placental Weight	Pearson Correlation	.000			1	
	Sig. (2-tailed)	.000				
	N	73	73	73	73	73
Gender	Pearson Correlation	.857				1
	Sig. (2-tailed)	.853				
	N	73	73	73	73	73
	N	73	73	73	73	73

4.5 Comparison between CD34+cell yield in normal deliveries and caesarean deliveries

There was positive relationship between CD34+ Cells and Method of delivery, CD34+cells are affected by the method of delivery that a mother or doctor may opt to use during pregnancy (p= 0.028). The analysis was done at 95% (0.05) confidence level

and a significant relationship of p = 0.028 and r = 0.033. The findings do reflect that method of delivery (Vaginal or Caesarean) does affect the yield of CD34+ Cell during pregnancy Table 4.5

Table 4.5: Relationship between CD34+method of delivery

Correlations			
		CD34+	**Method of Delivery**
CD34+	Pearson Correlation	1	.028
	Sig. (2-tailed)		.033
	N	73	73
Method of Delivery	Pearson Correlation	.028	1
	Sig. (2-tailed)	.033	
	N	73	73

CHAPTER FIVE

DISCUSSION

5.1 CD34+ cell yield/ml of umbilical cord blood

Cord blood is a good source of hematopoietic progenitor cells with potential for reconstitution. These cells are essential for the treatment of most hematologic neoplasias. Umbilical cord blood is also largely employed as an alternative source of stem cells in the treatment of haemato-oncological diseases. Current results show that the success rate of purified umbilical cord blood engraftment is comparable to that obtained using bone marrow, and it is directly related to the number of pluripotent stem cells transplanted. (Watt *et.al* 2010) In this study we found that cord blood contains CD34+ cell in various absolute counts and percentages. The percentages ranged from 1%-3%, with most participants having 1.6-2.0% of CD34+/ml of blood, this agrees with several studies that majority of the percentage gating range from 1.5-2.0% (Glasser *et.al*,2015, Gluckman *et.al* 2000, Rosenau *et.al* 2012)

Several studies have shown the simplicity of umbilical cord blood collection, in addition to the lack of risk for both mother and newborn, low risk of graft-versus-host disease, it is a useful alternative of hematopoietic stem cells for transplantation to treat diseases of the blood and for genetic disorders.

Many factors have been suggested to influence the quantity of CD34+ Cell derived from the umbilical cord blood, and that may be responsible for the variations in the reported results. Among these factors are the birth weight, sex of the newborn and maternal age which may have an effect on the concentration of CD34+ cells and this factors were among the factors done in our study.

5.2 Correlation between CD34+Cell Yield to Gestation Age, Birth Weight, Placental Weight and Gender.

In our study, significantly higher CD 34 + cell counts were associated with birth weights above 3500g. To reinforce these findings, other studies reported that greater birth weight positively influenced CD34+cell concentration. In order to determine which obstetrics factors influence the quality of umbilical cord blood, (Mancinelli *et al*., 2006) showed that a higher birth weight was associated with better quality of umbilical cord blood in relation to the CD34+ counts. Other authors also reported that higher birth weight factor was associated with increased CD34+ counts; findings agree with this study. Thus, the greater the birth weight, the more the CD34+ count. High birth weight had higher concentration of CD34 cells, this associations was highly significant with a $p < 0.00$.

According to the results of this study, the gestational age positively influenced the CD34 + cell concentration. The greater the gestational age, the more the CD 34+ cells. According to Askari *et al*.2005, in order to demonstrate which variables affect the t main quality parameters of umbilical cord blood, showed that a gestational age of over 36 weeks was a predictor for a larger volume of blood hence more cells, similarly, findings from this study indicates high gestation period gives more CD34+ cell counts. From this study, high CD34+ cell concentration was observed in those infants with a gestational age of more than 36 weeks. Some authors believe that with advancing gestational age and consequently with placental aging, the foetus would become hypoxic thus resulting in defence mechanisms. These mechanisms would be responsible for the increases in the number of hematopoietic cells in the cord blood.

To establish a CBB, the number of CD34+ cells in each cord blood unit (CBU) must be adequate, that's why neonatal and maternal factors should be considered when selecting CBUs.Many factors have been suggested to influence the quantity and quality of CD34+ Cell derived from the cord blood, and these may be responsible for the variations in the

reported results. Among these factors; the birth weight, sex of the newborn and maternal age which may have an effect on the concentration of the CD34+ cells.

Recent studies report that some variables affect the quality of the umbilical cord blood.Those related to maternal and fetal features such as placental weight, birth weight, gestational age (GA), route of delivery, gender of the newborn, among other things; thus research is being developed in this area to attempt to improve the cell levels hence increasing grafting success rates.

According to our findings, The more the placental weight the higher the CD 34+ count, The mean placental weight was 520 grams, It was observed that infants with high placental weight had more CD34+ cell concentration These findings agrees with (Nakagawa *et al,* 2004) who observed that higher percentage of CD34+ cells in the umbilical cord blood is related to high placental weight. Gender has no correlation with CD34+ cell counts according to our study and this is also observed in other studies (Jan R.H *et.al* 2008). There was no significant difference between baby boys and girls regarding this laboratory variable (CD34+cell concentration).

Many factors can influence the choice of using a single UCB stem cell donor for transplant including blood group typing, HLA typing, birth weight, neonatal gender, parity, prolonged first stage of labor and Apgar scores. Technical factors can also influence the choice of a single UCB stem cell donation such as CD34+ count, procedures for processing and storage time.

In Japan (Tokyo metropolitan area), retrospective analysis of UCB stem cell transplants showed that the most important positive predictors for success were first pregnancy of the donor and age above 30. Other factors such as birth weight, collected volume, CD34+ count and gestational age showed positive associations with TNC. Order of birth had a significantly negative association.

In Brazil, different cross sectional studies have been performed over the years to correlate maternal and fetal factors to cord blood as source of stem cells. An earlier study found that cord blood collection technique and newborn weight were positively correlated with the TNC count. Later, a different group demonstrated that birth weight and delivery route affect cord blood volume and TNC while gestational age affects the cord blood volume.

Carolinas Cord Blood Bank defined high-quality CBU as those with higher TNC count (>1.25 -109), and CD34+/CFU (colony forming units) in the upper quartile. They demonstrated the higher CD34+ were associated with interval from collection to processing <10 hours, gestational age (34-37 weeks), birth weight >3500 grams and CBU collected volume >80ml.

5.3 Comparison of the CD34+cell yield between normal deliveries and caesarean deliveries

When the route of delivery was analysed, it was seen that vaginal delivery was associated with higher CD 34+ cell count. It has been suggested that spontaneous labours are accompanied with a more CD 34+ cell counts. Statistically significantly higher CD 34+ cell concentration in the umbilical cord blood collected in the course of spontaneous labours was observed as compared with elective caesarean sections, this agrees with Sparrows study that suggests that a higher number of hematopoietic cells were observed in the umbilical cord blood after spontaneous labours (Sparrow *et al.*, 2002). Labour in vaginal delivery is an experience that has been associated with higher levels of various foetal leukocytes, including monocytes (Glasser *et.al* 2015) and granulocytes (Gluckman *et.al* 2000). During labour, the activation of cytokines has also been found in systemic maternal circulation (Watt *et.al* 2010).

In summary, Proper donor selection may predict better product and hence make the system more cost-effective by increasing the probability of processing those units that

are more likely to be banked. Cord blood banks with limited resources can focus on variables that produce better CD34+cell counts, which in this study were higher birth weight of the baby, heavier placenta, more gestation period and vaginal mode of delivery. Hence, we can cost-effectively increase our cord blood donor database and subsequently cord blood transplants be performed in the country.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Umbilical cord blood (UCB) is a rich source of hematopoietic stem cells that are CD34+. While these were previously being discarded, they can be collected shortly after birth with great capacity to reconstitute the hematopoietic system. More so, there is simplicity in collection of umbilical cord blood, lack of risk for mother and newborn, low risk of transmitting infectious diseases adds to its list of advantages.Furthermore,it is a useful alternative of hematopoietic stem cells for transplantation to treat diseases of the blood and genetic disorders.

2. The CD34+ cell yields in corded blood have a positive correlation to birth weight, placental weight, vaginal delivery, gestation age and a direct negative outcome with foetal gender. The successful engrainment of transplanted cells depends on both, the quantity and quality of stem cells. It is very important to obtain a large amount of good umbilical cord blood to improve its usefulness in transplantation.

3. Low yields were observed in mothers undergoing caesarean sections. However, If a cesarean delivery is performed, cord blood sampling is more efficacious if performed before delivering the placenta. This collection method seems beneficial and safe and might therefore be preferably used for cord blood stem cell banking and transplantation.

6.2 Recommendations

1. Corded blood from mothers whose gestations are above 36 weeks, foetus with birth weights above 3000gms, placental weight above 450gms will give better CD34+ stem

cell yields. Cell dose is the main limiting factor for the widespread adoption of UCB as a source of hematopoietic progenitors. For this reason it is valuable to optimize donor selection, collection method and many obstetric factors to achieve high-nucleated cell dose.

2. Vaginal deliveries would give better yields of CD34+ stem cells that may be of benefit for medical interventions. In the course of spontaneous labours, the collected umbilical cord blood has more cells as compared to elective caesarean sections.

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APPENDICES

Appendix I: Informed Consent form

Title of Project: Correlation of Cd34+Stem Cell Yield Derived from Umbilical Cord Blood with Birth Weight, Gender, Placental Weight and Gestation Age at Kenyatta National Hospital In Kenya.

Investigators:

1. Murei Jepkosgei Vibian, Principal Investigator (Masters Student, JKUAT)

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Introduction

My name is Murei Jepkosgei Vibian, and I am a student at the Jomo Kenyatta University of Agriculture and Technology. This study intends to determine the appropriate selection criteria in obtaining stem cells in cord blood. The findings of this study will be extremely useful especially as part of the steps we are taking to establish a cord blood centre in our region which will help treat most malignancies like leukaemia.

We will summarize our findings from this study and disseminate it to various

stakeholders including Ministry of Health, World Health Organization and others. The findings of this study will also be published in an international peer reviewed journal. The Kenyatta national hospital ethical review committee, who are responsible for conducting such reviews at national level, have approved this study.

RESEARCH PROCEDURE

WHAT IS A RESEARCH STUDY?

Research studies help us learn new things. We can test new ideas. First, we ask a question. Then we try to find the answer. This paper talks about our research and the choice that you have to take part in it. We want you to ask us any questions that you have. You can ask any time.

Important things to know…

- You get to decide if you want to take part.
- You can say 'No' or you can say 'Yes'.
- No one will be upset if you say 'No'.
- If you say 'Yes', you can always say 'No' later.
- You can say 'No' at any time.
- We would still take good care of you no matter what you decide

WHY ARE WE DOING THIS RESEARCH?

We are doing this research to determine the appropriate selection criteria in obtaining stem cells in cord blood.

- If you join the study, the researchers will do a test on your baby's cord blood.
- The blood test may show the researchers that certain criteria should be used to select cord blood donors.

WHY AM I BEING ASKED TO JOIN THE STUDY? You never know if your baby will be born with a malignancy, this study is part of the steps we are taking to establish a cord blood centre in our region which will help treat most malignancies like leukaemia.

WHAT WILL HAPPEN IF I AM PART OF THIS STUDY?

If you decide to be in the research, we would do the following things: The nurse will be able to get your baby's cord blood and placenta after you have safely delivered your baby. She/he will collect the cord blood after clamping of the cord and I assure you that the procedure will not in any way affect your baby and you as the mother. We will be able to use the cord blood to carry out the study which will be one of the stepping stones towards establishing a cord blood bank where cord blood will be stored for future use and will be used to save lives.

COULD BAD THINGS HAPPEN TO ME AND MY BABY IF I JOIN THIS RESEARCH?

No there will be no bad thing that can happen to you and your baby.

You can say ‘no’ to what we ask you to do for the research at any time and we will stop.

WILL I LEARN ABOUT MY RESULTS?

No, we will not be able to communicate the results to you.

WHAT ARE THE COSTS TO ME?

You will not pay for the study tests.

You will not be paid for being in this study.

WHAT IF I WANT TO QUIT THE STUDY?

If you don’t want to be in the study, you don’t have to be.

It is also OK to say yes and change your mind later. You can stop being in the research at any time. If you want to stop, please tell the researchers.

WILL THIS STUDY HELP ME? Being in this study may not help you for now but later after establishing a cord blood bank, it will help you or your siblings and other people at large.

WHO WILL KNOW THAT I AM PART OF THE STUDY?

If you are in the study, your information will be labelled with a number instead of your name. The paper with your name that tells your number will always be locked up so that only the study team will know your number.

MAY I ASK QUESTIONS?

You can ask the study team questions about this study at any time. You can call Murei Vibian anytime. Using the number 0723685374, E-mail kosymurei@yahoo.com

DO YOU WANT TO BE IN THE STUDY?

If you want to be in the research after we talk, please write your name below. We will write our name too. This shows we talked about the research and that you want to take part.

Name of Participant _____

Printed Name of Researcher

_____

Signature of Researcher

_____

Appendix II: Data Collection Sheet (To Be Filled by the Researcher)

Age at first menstrual period. _____

How many pregnancies have you had? ____

How many children have you given birth to? _____

Age at first delivery? _____

Have you ever convulsed during your pregnancy? _____

Are you diabetic? _____

Are you hypertensive? _____

What was your last haemoglobin level? _____grams/dl

Have you had any infections during your pregnancy? _____ if yes, name them:

_____

_____

Weight of the mother (if known) _____kg

Date of last menstrual period? _____

Sex of the child Female___ Male_____

Birth weight of the child_____grams

Placental weight_____grams Gestation Age _____

Appendix III: Standard Operating Procedure for Flow Cytometry

Principle

When whole blood is added to the reagent, the fluochrome-labelled antibodies in the reagent bind specifically to Leucocyte surface antigens.

During acquisition, the cell travels past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals detected by the instrument, provide information about the cell size, internal complexity and relative fluorescence intensity

METHOD

Before use, verify that BD Trucount bead spelled is intact and within the metal retainer.

1. Label the BD Trucount tube with the patient's number.

2. Pipette 20ul of BD tritest CD34 reagent into the bottom of the tube (just above the stainless-steel retainer).

3. Add into the tube 50ul of well mixed EDTA coagulated whole blood (Dispensing into the bottom of the tube with reverse pipetting).

4. Cap the tube and vortex gently to mix.

5. Incubate for 15 minutes in the dark at room temperature.

6. Add 450Ul BD FACS lysing solution to the tube.

7. Cap the tube and vortex gently to mix.

8. Incubate for 15minutes in the dark room temperature.

9. The sample is now ready to be analysed on the flow cytometer.

NB: If the samples cannot be analysed immediately, they must be kept in darkness at room temperature for up to 48 hours.

Reference

University of Nairobi Standard Operating Procedures No: 2