This supplement contains the following items:

1. Original protocol (page 1-9)

2. Original statistical analysis plan (page 10-14)

(No amendments have been made from the initial study plan)
Effect of timing of umbilical cord clamping on anaemia at 8 and 12 months in late pre-term and term infants; a randomized-controlled trial

Ashish KC¹,²,*
Email: ashish.k.c@kbh.uu.se

Mats Målqvist¹
Email: Mats.malqvist@kbh.uu.se

Nisha Rana³
Email: nishaarana@gmail.com

Linda Jarawka Ranneberg⁴
Email: lindaranneberg@gmail.com

Ola Andersson¹
Email: ola.andersson@kbh.uu.se

¹ International Maternal and Child Health, Department of Women’s and Children’s Health, Uppsala University, Uppsala, Sweden

² United Nations Children’s Fund (UNICEF), Kathmandu, Nepal

³ Paropakar Maternity and Women’s Hospital, Kathmandu, Nepal

⁴ Department of Paediatrics, Hospital of Halland, Halmstad, Sweden

* Corresponding author. International Maternal and Child Health, Department of Women’s and Children’s Health, Uppsala University, Uppsala, Sweden

Abstract

Background

Delayed cord clamping at birth has shown to benefit neonates with increased placental transfusion include higher haemoglobin concentrations, additional iron stores and less anaemia later in infancy, higher red blood cell flow to vital organs and better cardiopulmonary adaptation. As iron deficiency in infants even without anaemia has been associated with impaired development, delayed cord clamping seems to benefit full term infants also in regions with a relatively low prevalence of iron deficiency anaemia. In Nepal, children age 6-17 months have high anaemia prevalence (72-78%). The objective of the proposed study is to evaluate the effects of delayed and early cord clamping on anaemia (and haemoglobin level) at 8 and 12 months, ferritin at 8 and 12 months, bilirubin at 2-3 days, admission to NICU or special care nursery and development at 12 and 18-24 months of age.
Methods/Design

Randomized controlled trial comparing delayed and early cord clamping will be implemented in the hospital. The study will be conducted at the Paropakar Maternity and Women’s Hospital, Nepal. Pregnant woman of gestational age 34-41 weeks who deliver vaginally will be included in the study. The interventions consist of delayed clamping of the umbilical cord (≥180 seconds after delivery) or early clamping of the umbilical cord (≤60 s). At 8 and 12 months of age, infant’s iron status and developmental milestones will be measured.

Discussion

The trial is important to perform as although strong indications exists on the effect of delayed cord clamping on anaemia at 8 to 12 months of age, it has not been evaluated by a randomized trial. By the proposed study, outcome as well as safety effects will be analysed, and results might not only contribute to practice in Nepal, but also to the global community, in particular low income countries with a high prevalence of iron deficiency anaemia.

Trial registration

NCT02222805

Background

At the time of birth, the infant is still attached to the placenta via the umbilical cord. The infant is usually separated from the placenta by clamping the cord with two clamps. This task takes place during the third stage of labour, which is the period of time from the birth of the infant to the delivery of the placenta.

Active management of the third stage of labour has been described in a recent World Health Organization (WHO) report as the “cornerstone” of obstetric and midwifery practice during the latter part of the 20th century. Active management has involved the clinician intervening in the process through three interrelated processes: the administration of an uterotonic drug; early cord clamping and cutting; and controlled traction of the umbilical cord.

Early cord clamping has been generally advised to be carried out in the first 30 seconds after birth, regardless of whether the cord pulsation has ceased. Due to evidence shown the last decade, recent guidelines for management of the third stage of labour no longer recommend immediate cord clamping but changes in practice is questioned and policies on hospitals are rare.

Delayed clamping allows time for a transfer of fetal blood in the placenta to the infant at the time of birth. This placental transfusion can provide the infant with an additional 40% more blood volume. The amount of blood returned to the infant depends on when the cord is clamped and at what level the infant is held prior to clamping. Neonatal benefits associated with this increased
placental transfusion include higher haemoglobin concentrations, additional iron stores and less anaemia later in infancy, higher red blood cell flow to vital organs and better cardiopulmonary adaptation\textsuperscript{1,9,10}.

Delayed cord clamping has, however been linked to an increase in the incidence of jaundice which, in severe cases, could have longer term effects on the health and development of the infant\textsuperscript{1,11}.

Previous studies performed by the principal investigator in a high-income country has shown that delayed cord clamping, compared with early clamping, resulted in reduced prevalence of neonatal anaemia\textsuperscript{12}. Furthermore delayed, cord clamping improved iron status and reduced prevalence of iron deficiency (ID) at 4 months of age without demonstrable adverse effects\textsuperscript{12-14}. As ID in infants even without anaemia has been associated with impaired development\textsuperscript{15,16}, delayed cord clamping seems to benefit full term infants even in regions with a relatively low prevalence of ID anaemia\textsuperscript{12}.

The improved iron stores at four to six months after delayed cord clamping suggests that ID anaemia could be reduced at eight to twelve months of age, but this could not be shown in the principal investigators latest study\textsuperscript{17}, possibly due to small sample size and low frequency of ID anaemia. Although ID anaemia is rare (3-9 \%) in high-income countries\textsuperscript{18}, the negative impact on children’s health and development should not be underestimated. No randomized trial has evaluated the effect of delayed versus early cord clamping on infants after 6 months of age in a low income country with high prevalence of iron deficiency and anaemia. As anaemia is associated with extensive health effects, such as stunting, fatigue and impaired neurodevelopment\textsuperscript{19} reducing anaemia in infants is an urgent need in a global perspective. In a recently published observational study from Peru, anaemia at eight months of age was evaluated at infants born before and after a hospital change of regime from early to delayed cord clamping. The study resulted in a significant reduction of anaemia by 16\% (from 75 to 59\%) as well as a significant higher level of haemoglobin\textsuperscript{20}.

In Nepal, children age 6-17 months have high anaemia prevalence (72-78\%)\textsuperscript{21} Approximately 50\% of all anaemia among pre-schoolers can be contributed to ID\textsuperscript{22}. By performing the planned study in a country with high anaemia prevalence, we can reduce sample size dramatically and still be able to detect significant effects on haemoglobin levels and neurodevelopment.

**Study Objective**

To evaluate the effects of delayed and early cord clamping on

1. Anaemia (and haemoglobin level) at 8 and 12 months
2. Ferritin at 8 and 12 months
3. Bilirubin at 2-3 days
4. Admission to NICU or special care nursery
5. Development at 12 and 18-24 months of age.

**Primary outcome**

The primary outcome will be infant haemoglobin 8 months of age.
Secondary outcomes
The secondary outcomes will be

- Haemoglobin at 12 months
- Ferritin at 8 and 12 months; definition iron deficiency as ferritin less than 12 μg/L
- Iron deficiency anaemia at 8 and 12 months, defined as both ferritin and haemoglobin below the respective cut offs

Other outcomes will be hyperbilirubinemia at discharge, breast-feeding and morbidity during the first six months of life and psychomotor development at 12 months and development evaluated by The Bayley Scales of Infant and Toddler Development at 18-24 months.

Methods

Trial design and Participants
Randomized controlled trial with allocation ratio 1:1 comparing delayed and early cord clamping will be implemented in the hospital. The study will be conducted at the Paropakar Maternity and Women’s Hospital, Nepal.

In the hospital there are two separated delivery departments, high risk-labour room (LR) and low risk-Maternal and Neonatal Service Center (MNSC). At admission, according to the hospital protocol, the pregnant women are screened by an obstetrician who made the decision to which department the women will be transferred.

The hospital criteria for admission to MNSC are-uncomplicated pregnancies, no complication at the time of admission and healthy mothers (no clinical history of hypertension, infection, diabetes, chronic medical condition), expected vaginal delivery, gestational age between 34 and 41 weeks and singleton pregnancy.

Women will be eligible to participate in the study if they are assigned to MNSC. The exclusion criteria will be serious congenital malformation, syndromes, or the other congenital disease that could affect the outcome measures. Written consent will be obtained from the women who were eligible and willing to participate

Inclusion
As the pregnant woman arrives at the hospital, she will receive written information about the study in the reception. Surveillance officers (SO) will then approach the woman and ask for her consent to participate in the study.

After consent, the SO help out to register the information needed in the protocol. The woman labour is after this managed by the hospitals ordinary routine until she is transferred to the delivery where the SO will pair the women with a sealed, numbered, opaque envelope containing the treatment allocation and show this for the midwife.
**Intervention**
When delivery is imminent (expected within 10 minutes), the midwife will open a sealed, numbered, opaque envelope containing the treatment allocation. The interventions consist of delayed clamping of the umbilical cord (≥180 seconds after delivery) or early clamping of the umbilical cord (≤60 s). The SO will measure the time from complete delivery of the baby to the first clamp on the umbilical cord with a stopwatch. All other aspects of obstetric care will be managed according to standard practice at the hospital. In both groups, oxytocin will be given to the mother after the umbilical cord is clamped. All staff in the delivery unit will be trained in the study procedures before the trial started.

**Follow-up**
After the delivery, the babies will be cared for according to clinical routines, and early breast feeding will be encouraged. As part of the study, the midwife will assess the infant at 1 and 6 hours, whether the baby had been breastfed and the presence of respiratory symptoms (that is, respiratory rate >60 breaths/minute, presence of nostril flaring, grunting, or intercostal retractions).

Infants will stay at the postnatal ward with their mothers for two or three days, except for well babies whose mothers preferred to leave the hospital earlier and infants who requires admission to the neonatal unit.

Monthly up to 12 months of age, a SO will call up the family and ask questions regarding infections, breast-feeding and immunizations.

At 8 and 12 months of age, infants will be scheduled for a follow-up visit including blood sampling (complete blood count, iron status, and C reactive protein) and weight and length measurements. Venous blood sampling will be performed. Parent will be assisted in answering the Ages & Stages Questionnaire at 12 months of age.

The Bayley Scales of Infant and Toddler Development, Third Edition will be used to examine children at 18-24 months of age.

Physicians performing neonatal examinations, staff members responsible for collection of blood samples and background data, and laboratory staff performing analyses of blood samples will be blinded to each infant’s allocation group.

**Additional data collection**
The following information will be collected from maternal healthcare records: background information of the mothers, parity, weight, previous obstetric history, current pregnancy history, clinical progress during delivery, babies weight, gestational age, Apgar score.

**Sample size**
The sample size for the primary outcome at eight months was estimated to find a difference of 15% (70% versus 55%) in the prevalence of anaemia between the two randomization groups with a power of 80% and a type I error rate of .05. Using Fisher's exact-test to analyze outcome data a group size of 176 would be needed. Taking into account an attrition rate of 35% we calculate to include 270 newborns in each group, i.e. a total of 540.
Timeline

The study will be initiated at October 2, 2014 with the expected enrollment of the 540 participants within 45 to 60 days of the start of the project.

Data management

The surveillance officer will fill up and assess records. The two research managers will verify all record forms with that from its primary source. A data entry officer will re-check them for discrepancies before entering the data in computers. The quality control team from Uppsala University will provide oversight to ensure quality of data collection and to avoid data loss a protocol for data tracking system will be followed.

The Census and Survey Processing System (CSPro), a public domain software package developed and supported by the U.S. Census Bureau and ICF Macro, will be used for quality data management. CSPro is interfaced with IBM SPSS Statistics 22 (originally, Statistical Package for the Social Sciences) which will be used for statistical analysis, data management (case selection, file reshaping, creating derived data), and data documentation. Hard copies of records will be stored in a filing system in a secure room. Data will be checked for accuracy, consistency, and completeness in both the CSPro and SPSS. An analysis plan will be developed in accordance with the reporting guidelines. A profile and a comparison of key variables between cases and referents at baseline will be presented.

Ethical considerations

All research involving newborn infants and small children need careful ethical considerations, mainly since the subjects themselves can’t agree to whether they want to participate in the study or not. In particular, research that is not immediately of benefit for the patients needs ethical consideration. The included infants are healthy full-term infants undergoing umbilical cord clamping, which is a standard procedure after birth. The possible benefit of the intervention (delayed cord clamping) of increasing iron stores and preventing infant anaemia is estimated to be higher than the risk of probable adverse effects, such as hyperbilirurubinaemia. Ethical approval has been sought and obtained from Nepal Health Research Council (Reg no. 76/2014) on 5 June 2014. Written informed parental consent will be obtained before the intervention, and parents can withdraw from the study at any time without any need for explanations.

Discussion and Policy Implication

Reducing ID among infants is important, as it is associated with impaired neurodevelopment. With high global prevalence of infant anaemia, delayed cord clamping has the potential to reduce infant anaemia and thereby improve infants’ and children’s health and development. In crude numbers, a reduction by 10% would mean an annual reduction of 60,000 infants with anaemia in Nepal. The trial is important to perform as although strong indications exists on the effect of delayed cord clamping on anaemia at 8 to 12 months of age, it has not been evaluated by a randomised trial. By the proposed study, outcome as well as safety effects will be analysed, and results might not only contribute to the practice in Nepal, but also to the global community, in particular low income countries with a high prevalence of ID anaemia.
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Authors’ contributions

AK, MM and OA conceptualized and designed the study. AK and OA drafted the study protocol manuscript. All authors provided intellectual input and approved the final manuscript.

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References

Statistical Analysis Plan

Regarding trial:

*Effect of timing of umbilical cord clamping on anaemia at 8 and 12 months and later neurodevelopment in late pre-term and term infants; a facility-based, randomized- controlled trial in Nepal*

Author: Ashish KC, Mats Målvist, Ola Andersson
**Introduction**

The investigators plan a study to randomize 540 children in Nepal to early (≤30 seconds) or late (≥180 seconds) clamping of the umbilical cord at birth. The children will be followed with blood tests (hemoglobin and ferritin) at 8 and 12 months of age, and their development is evaluated by questionnaire (Ages & Stages Questionnaire) at 12 months of age, and by testing (Bayley -III) at 18-24 months of age. By implementing the project in a country with a high proportion of anemia at one year of age (about 75%), we can reduce the number of children in the study and still achieve significant results.

Iron deficiency is a global health problem and causes anemia and impaired neurodevelopment in children. Anemia is estimated by WHO to occur among 25% of all children before school age, and the corresponding figure in Europe is 3-9%.

By waiting 3 minutes to clamp the cord after birth, a large part of the child's blood volume remaining in the placenta is transfused over to the child's body. Research shows that the neonate’s blood volume can increase by about 40% and this blood contains 3 to 4 months' supply of iron. In Sweden, we have shown that late clamping of the umbilical cord could reduce iron deficiency in children at four months of age by 90%. Globally, most countries practice early cord clamping and the child is deprived of the placental blood transfusion. The hypothesis of the study is that by delaying the clamping of the umbilical cord, anemia at 8 and 12 months will be reduced an this in turn will be beneficial for the childrens development.

**Data source:**

The SO will fill up and assess entered into the ‘Data collection tool’ (Appendix I). The research managers will verify all record forms with the primary source of data. A data entry officer will re-check them for discrepancies before entering the data in computers. The quality control team from Uppsala University will provide oversight to ensure quality of data collection and to avoid data loss. A protocol for data tracking will be followed.

**Analysis objectives**

To evaluate the differences between the intervention groups ‘delayed cord clamping’ and ‘early cord clamping’ in hemoglobin and ferritin at 8 and 12 months of age, by comparing means and by comparing the categorical variables ‘anemia’ (hemoglobin < 110 g/L) and ‘iron deficiency’ (ferritin < 12 µg/L).
Sample size

The sample size calculation is based on examining the effect of cord clamping on anemia at eight months of age. The national prevalence of anemia was 70% at eight months. To find a difference of 15% (from 70% to 55%) in prevalence between the treatment groups with 80% power and 0.05 type I error, 176 infants are needed in each group, and allowing for 35% attrition we decided to allocate 270 in each treatment group.

Analysis populations

This will be a randomized control trial in a hospital of Nepal with two parallel groups (1:1 ratio), delayed cord clamping (≥180 s) and early cord clamping (≤60 s).

Women will be eligible to participate in the study if they are assigned to the low risk Maternal and Neonatal Service Centre (MNSC) with the admission criteria: uncomplicated pregnancies, no complication at the time of admission and healthy mothers (no clinical history of hypertension, infection, diabetes, chronic medical condition), expected vaginal delivery, gestational age between 34 and 41 weeks and singleton pregnancy. The exclusion criteria will be serious congenital malformation, syndromes, or the other congenital disease that could affect the outcome measures.

Outcome measures and covariates

Primary Outcome Measures:

- Hemoglobin [ Time Frame: 8 months plus/minus one month ], analyzed by comparing means and as a categorical variable, defining anemia as Hemoglobin < 110 g/L

Secondary Outcome Measures:

- Hemoglobin [ Time Frame: 12 months plus/minus one month ], analyzed by comparing means and as a categorical variable, defining anemia as Hemoglobin < 110 g/L

- Ferritin [ Time Frame: 8 months plus/minus one month ], analyzed by comparing means and as a categorical variable, defining iron deficiency as Ferritin < 12 μg/L

- Ferritin [ Time Frame: 12 months plus/minus one month ], analyzed by comparing means and as a categorical variable, defining iron deficiency as Ferritin < 12 μg/L
• Iron deficiency anemia [Time Frame: 8 months plus/minus one month] Defining iron deficiency as a combination of Hemoglobin < 110 g/L and Ferritin < 12 μg/L

• Iron deficiency anemia [Time Frame: 12 months plus/minus one month] Defining iron deficiency as a combination of Hemoglobin < 110 g/L and Ferritin < 12 μg/L

• Bilirubin [Time Frame: 2 days] Measured by a transcutaneous method.

Covariates
Baseline or follow-up data that are not randomly distributed between treatment groups and significantly correlated with primary and secondary outcomes.

Altitude adjustment
Before making descriptive statistics and group comparisons, a correction of 3.2 g/L will be subtracted from each of the individual hemoglobin results to adjust for the altitude of Kathmandu of 1400 meters using the ‘CDC Hemoglobin adjustment method’ = -0.32 \times (\text{altitude} \times 0.0032808) + 0.22 \times (\text{altitude} \times 0.0032808)^2.

Expected transformation of ferritin
Ferritin is known to have a skewed distribution, and will be log_{10} transformed before analyzed as a continuous variable.

Handling of missing values
If attrition is higher than expected, the Multiple Imputation method in SPSS will be used. Variables included in the model as predictors are those possible to include from baseline and follow-up data: time to clamping, mother’s age, previous pregnancies, gestational age, birth weight, sex, and time from delivery to discharge in hours. Age in days, hemoglobin, and ferritin at eight months can be entered as both dependent and predictive variables while age, hemoglobin, and ferritin at 12 months of age, are entered as dependent variables. Imputation will be made five times.
Statistical procedures

All variables will be assessed for quality of data; dates, outliers and other possible unclear data checked with the protocol and if necessary, hospital records.

To evaluate differences between treatment groups, unpaired two-tailed t-test will be used for variable with normal distribution; categorical variables are compared between groups using Fisher’s exact test. A P value < 0.05 will be considered significant for all tests. All data will be analyzed and presented as by intention to treat.

Baseline date will be examined for correlations with the outcome variables using the Pearson (for normally distributed variables) or the Spearman correlation method, and by a scatterplot.

Sensitivity analysis

To control for baseline data that are not randomly distributed between treatment groups and significantly correlated with primary and secondary outcomes, MANOVA or logistic regression analysis will be used as appropriate to examine if adjusted analysis will render results different from unadjusted analysis. Also age at blood sampling at 8 and 12 months will be controlled for in the same manner.

To control for protocol adherence, all statistical analysis will also be performed including only infants handled according to their assigned allocation group. Results deviating from the intention to treat analysis will be reported.

All outcome measures had pre-defined cut-offs, and we do not plan to analyse the sensitivity to different cut-offs.

To control for missing data, multiple imputation (five times) will be done.