EFFECT OF UMBILICAL CORD MILKING COMPARED TO DELAYED CORD CLAMPING IN TERM INFANTS, A RANDOMIZED CONTROLLED TRIAL

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May 2017

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Abstract

Introduction

Delayed cord clamping at delivery is currently recommended to improve early neonatal haemoglobin levels. It carries an increased risk of postpartum haemorrhage and prolongs the operative time at caesarean sections. An alternative could be milking of the cord with early clamping to achieve similar increment in haemoglobin levels. Although effects of cord milking had been assessed in preterm neonates such evidence is sparse for term neonates.

Objectives

To evaluate the effects on neonatal haemoglobin, bilirubin, haemodynamic parameters and maternal blood loss in term infants following umbilical cord milking (UCM) compared to delayed cord clamping (DCC) during elective caesarean sections.

Methods

Sixty term pregnant mothers undergoing elective caesarean section in Teaching Hospital Kandy, from 1st February to 31st March 2015 were randomized to delayed cord clamping and cord milking. Control group had cord clamped after 2 minutes of delivery or once the cord pulsations had ceased. The intervention group had the umbilical cord milked towards the umbilicus of the baby in a standard method soon after delivery. Neonatal haemoglobin, bilirubin, haemodynamic parameters and maternal blood loss were assessed within 48 hours of delivery.

Results

27 subjects underwent DCC while 33 subjects underwent UCM. Main indications for the caesarean deliveries were past section (46.7%), failed induction (11.7%), past bad obstetric history (10.0%), maternal medical conditions (8.3%) and primipara with short stature (8.3%). All caesarean sections were performed under spinal aneasthesia. Control and the interventions groups were equally matched.

The mean haemoglobin value of the neonates in the control group was 17.364g/dl (±2.44) while the intervention group had a mean value of 17.642g/dl (±1.97). There was no statistical significant difference in haemoglobin values in 2 groups (p=0.67). The mean total bilirubin values in the control and intervention groups were 123.0mmol/l (±34.99) and 111.8mmol/l (±47.61) respectively with no significant difference (p=0.685). All neonates investigated had birth APGAR of 10 at 5 minutes. Mean maternal haemoglobin drop was 1.27mg/dl (±0.95) and 1.04 mg/dl (±0.68) (p=0.37) after DCC and UCM respectively.

Conclusions

UCM is an effective and a safe alternative to DCC in term infants during elective caesarean deliveries.

Key words – Umbilical cord milking, Delayed cord clamping, Term, Haemoglobin, Bilirubin, Blood loss, Caesarean

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Abbreviations

Bpm – Beats per minute

DCC – Delayed cord clamping

ECC – Early cord clamping

Hb – Haemoglobin

UCM – Umbilical cord milking

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Acknowledgement

This research for comparison of different cord clamping methods was refined at the early study day programs conducted by Prof Malik Goonewardena from Department of Obstetrics and Gynaecology, Teaching Hospital Mahamodara. His teaching on research methodology was instrumental in initial development of the research proposal. Prof. Kapila Gunawardena who was my initial supervisor guided me in further refinements as well as helping with ethical clearance.

The research was carried out with the kind permission and supervision of Dr. Sardha Hemapriya at Kandy teaching hospital. Midwifery, nursing and the minor staff of the ward 5 Teaching Hospital Kandy as well as all the staff of operation theatre C helped me immensely in conducting the research. Dr. Damayantha Wattuhewa and Dr. Achintha Dilshan Dissanayake carried out all the caesarean sections in addition to my self. They underwent the training for the research and took every effort to adhere to research protocol when performing the surgeries. All the Intern House officers at ward 5, Teaching Hospital Kandy and Dr. Lavanya Chethani Devasurendra who was the Resident House Officer at Surgical Intensive Care Unit helped in all steps of the research. The laboratory staff at the Teaching Hospital Kandy gave their generous support in blood sample analysis without a delay.

During writing of the dissertation Dr. Lakshman Kariyawasam, consultant Obstetrician and Gynaecologist, Castle Street Hospital for women and Dr. Anuruddha Abeygunasekara, Consultant Urologist at Colombo South Teaching Hospital gave their valuable ideas and time.

I would like to thank all of them since if it were not for them this effort would not have been a reality.

Section – 1

Background and justification

Anaemia in children is a common problem in Sri Lanka. According to the National Demographic and Health Survey, which was conducted in 2006-7 the prevalence of anaemia among children under 5 years was 32.6%[1]. The main contributor for childhood anaemia is iron deficiency. Iron deficiency can be detrimental to the development of neonates even progressing to childhood. Improving haemoglobin by 1g/dl will lead to significant reduction in perinatal mortality and mental retardation[2].

The amount of blood in the feto-placental circulation of a term neonate (including the placenta and the fetus with the umbilical cord) is estimated to be 120ml/kg. At the time of delivery this amount distributes in a ratio of 2:1 to the fetus and the placenta respectively, if the umbilical cord was clamped immediately. Therefore with immediate cord clamping the amount of blood in newborn infant's circulation is estimated to be 78 ml/kg. This will correspond to a venous hematocrit of about 48 percent. Alternatively if the cord clamping is delayed approximately for about 3 minutes this distribution will be more towards the fetus in a ratio of about 7:1. Delayed clamping for 3 minutes will result in mounting of the blood in the fetal circulation up to 105ml/kg[3, 4].

Delayed cord clamping against early cord clamping had been the subject for several randomized studies and Meta analysis over the years. The conclusions drawn from these studies is that there is a significant increase in haematocrit as well as haemoglobin values in early neonatal life in the delayed cord clamped neonates compared to the early cord clamped neonates. But this effect was not apparent with regards to the neonatal haemoglobin values by 4 months of age, where there is no significant difference in haemoglobin levels between the two groups by this time. But the delayed cord clamped infants still had higher iron stores reflected by higher serum ferritin levels at 4 months of age[5, 6]. Therefore it was concluded that delayed cord clamping is a low cost intervention for preventing neonatal anaemia[7].

Nevertheless delayed clamping is also associated with adverse maternal and fetal outcomes. Delayed cord clamping is conflicting with the concept of active management of third stage of labour which includes early cord clamping, controlled cord traction and administration of intravenous oxytocin at the time of delivery of the baby. The active management of third stage of labour has shown to significantly reduce primary post partum haemorrhage as well as the average time of third stage of labour when compared to expectant management[8]. Therefore not adapting active management and delaying the cord clamping can lead to increase bleeding following delivery. Delayed cord clamping needs waiting for additional 2-3 minutes, which can prolong the operative time during caesarean sections. On the other hand due to this delay it may not be feasible to adapt this method at all deliveries since some conditions warrant immediate cord clamping and neonatal resuscitation. Sometimes these neonates are the most susceptible for low haemoglobin and blood transfusions that would be the most benefitted group by even a slight increment in their blood volumes. Also there can be adverse effects of successful delayed clamping due to higher number of red blood cells in fetal circulation, namely polycythemia and later hyperbilirubinaemia which may warrant phototherapy.

The umbilical cord of a full term human neonate averages 50 to 70cm long and 2cm in diameter[9]. It is estimated to contain on average 108ml of blood. This is about one third of the blood volume in a term infant, which approximates to about 300ml[10]. Umbilical cord is easily accessible to the operator and if this 100ml of blood can be forced in to the fetus this will invariably improve the fetal circulation with minimal delay. As mentioned above most of the complications of delayed cord clamping are due to the delay of 2 to 3 minutes. Therefore forcing the blood in the umbilical cord towards the fetus by means of cord milking followed by early cord clamping may be an alternative to achieve higher haemoglobin levels in the neonate without the above mentioned adverse outcomes.

Umbilical cord milking reduces the delay in third stage as well as the operative time. There are several studying investigating the effect of umbilical cord milking on preterm infants. These studies have concluded that in preterm infants cord milking

significantly increase the immediate haemoglobin levels as well as the iron stores[11, 12]. But some have concerns in forcing the blood by milking since it can traumatize red blood cells, which lead to excess bilirubin delivered to a fetus.

A study done by Rabe at el in preterm infants comparing umbilical cord milking with delayed cord clamping, which is the current recommendation in management of third stage of labour showed comparable outcomes with regards to the early haemoglobin levels in the two groups[13].

Unfortunately there are no published studies comparing umbilical cord milking with the delayed cord clamping in term neonates. This study is based on the null hypothesis that there is no difference in umbilical cord milking compared to the delayed cord clamping in haemotological parameters in term neonates.

Section – 2

Literature review

Neonatal anaemia

The average venous haemoglobin in a neonate after 34 weeks is 17g/dl. When it comes to defining anaemia in the newborn it is defined as venous blood haemoglobin less than 13g/dl or capillary haemoglobin less than 14.5 g/dl. Although there are no demographic data on anaemia of the Sri Lankan newborns it is a common problem among Sri Lankan children. According to the Sri Lanka demographic and health survey in 2007, anaemia in children under the age of five years was 32.6%. The UNICEF report on maternal, newborn, child health and nutrition for survival and development, national profile Sri Lanka 2010 gives a figure of 25.5% for anaemia under 5 years in 2009[1].

Anaemia increases the risk of perinatal mortality as well as mental retardation. According to the chapter on anaemia by World Health Organization, increasing 1g/dl of haemoglobin will reduce the relative risk for perinatal mortality to 0.72, 95%CI 0.65–0.81 and for mental retardation to 0.78, 95%CI 0.70–0.86 [2]. The commonest reason for anaemia is iron deficiency. Iron deficiency in early childhood leads to development delay[14].

Total blood volume in the fetal circulation

Usher at el measured the blood volume of 27 neonates by using iodinated human. At the time of delivery the newborn infant was estimated to have a blood volume of 78ml/kg with a venous hematocrit of 48% [4].

Yao at el measured the distribution of blood between the placenta and the fetus at the time of delivery. Total amount of blood in the feto-placental unit was 120ml/kg, which was measured according to iodine dilution. With immediate clamping of the cord the distribution of the blood between the fetus and the placenta was in a ratio of 2:1. Delaying the cord clamping lead to an increase in the blood volume of the fetus, which increased up to a ratio of 8:1 with a 3-minute delay[3].

Wang who studied the vascular biology of the placenta described the dimensions of the umbilical cord in a full term human neonate. Accordingly the umbilical cord averages 50 to 70cm in length and the diameter is about 2cm [9]. Therefore the umbilical cord can accommodate a certain volume of blood from the feto-placental unit. Yamada at al measured the volume of umbilical cord blood obtained in 29 cesarean deliveries. The mean volume was 103.9 ml (+/- 33.6)[15]. Similarly McDonald at el in his studies gives an average of 108ml for umbilical cord blood. His estimate for the newborn's blood volume was 300ml [10]. So on average umbilical cord contains one third of the blood volume in the newborn.

Early cord clamping (ECC) and delayed cord clamping (DCC)

A study conducted in 2009 in Sweden investigated the difference between ECC and DCC. In this study the ECC group had the umbilical cord clamped within 10 seconds of delivery and the DCC group after 180 seconds of delivery. The DCC group had lower prevalence of neonatal anaemia at 2 days of age with only two (1.2%) anaemic infants in the DCC group verses ten (6.3%) in the ECC group (P=0.02). The number needed to undergo DCC to prevent one case of anaemia was 20. But this effect was not apparent at 4 months of age where infants showed no significant differences in haemoglobin concentration between the groups. However the infants subjected to DCC had 45% higher mean ferritin concentration (117 µg/L versus 81 µg/L, P<0.001) and a lower prevalence of iron deficiency (1 versus 10, P=0.01. Therefore the effects were apparent and beneficial till early infancy. There were no significant differences between the two groups with regards to postnatal respiratory symptoms, polycythaemia or hyperbilirubinaemia, which required phototherapy[5].

A meta analysis carried out in 2013 including 15 trials with over 3500 subjects concluded that DCC was beneficial compared to ECC for the neonate without any difference between maternal outcomes. When considering the neonatal outcomes haemoglobin values were significantly higher in delayed cord clamped group at 24 hours but the effect did not persist at the age of 3 to 4 months. Additionally the late clamping group had significantly less iron deficiency. Considering adverse effects to

the neonates the late clamped group required significantly more phototherapy (RR 1.61; 95% CI 1.04 to 2.43) although there was no significant difference in the occurrences of clinical jaundice. There was no difference in respiratory distress between the two groups as well as admissions to special care baby units. The maternal outcomes, which were considered, were post partum haemorrhage, severe maternal morbidity and mortality[6].

Hutten et al carried out a meta analysis that included 1912 term newborns. DCC group had 1001 newborns whose cords were clamped at least 2 minutes after birth and the ECC group had 911 infants whose cords were clamped immediately after birth. Benefits over the age of 2 to 6 months associated with late cord clamping were higher Haematocrit (mean difference 3.70%; 95% CI 2.00% to 5.40%), serum ferritin concentration (MD 17.89; 95% CI 16.58 to 19.21) and clinically important reduction in the risk of anemia (RR 0.53; 95% CI 0.40 to 0.70). But this late cord clamped group of neonates was at increased risk of experiencing asymptomatic polycythemia (RR 3.82; 95% CI 1.11 to 13.21)[16].

When cord is clamped after a delay of 5 minutes the blood volume in the newborn increased by 61% to 126 ml/kg. For a 3.5 kg infant the volume of placental transfusion was 166 ml. one-quarter of which occurred in the first 15 seconds, and one-half within 60 seconds of birth. Usher at al noted that stripping the umbilical cord 10 times during the 5 minutes did not further increase the volume of the transfusion[4].

Therefore late cord clamping is a low cost intervention for preventing neonatal anaemia[7]. It is recommended that every neonate born at resource poor settings should be considered DCC. The delay should be about 3 minutes[17].

Disadvantages of DCC

Five studies conducted from 1990 to 1998 were analyzed in a Cochrane review, which was published in year 2000. Four of the trials were of good quality. Compared to expectant management, active management reduced average maternal blood loss by 79ml (95% CI 94 to 64). Furthermore post partum haemorrhage of more than 500ml

was also significantly reduced by active management in the third stage of labour (RR-0.38, 95% CI 0.32 to 0.46). These effects were mainly due to reduction in the duration of third stage of labour (weighted mean difference -9.77 minutes, 95% CI -10.00 to -9.53) by employing active management in the third stage. There was no apparent advantages or disadvantages with either technique for the baby[18].

Inherent part of active management is early cord clamping. DCC will prolong the third stage of labour thus increasing the risk of haemorrhage. Therefore it is recommended to give uterotonics immediately after the delivery of the fetus even with delayed cord clamping[17].

DCC prolongs the operative time in caesarean sections by 2 to 3 minutes. Due to higher risk of haemorrhage this method cannot be adapted to all deliveries. Also certain neonates need early resuscitation due to antenatal and intrapartum complications. Delaying a few minutes can adversely affect the health of the newborn.

Umbilical cord milking (UCM)

Colozzi published a review article in 1954 in the New England Journal of medicine on different ways of umbilical cord clamping and its effects on neonatal haemoglobin levels. He stated that average haemoglobin value after early cord clamping was 14.85mg/dl. The respective value for the delayed cord clamping when arterial pulsations stopped was 17.38 mg/dl. Early cord clamping followed by milking the cord achieved even higher average neonatal haemoglobin values mounting to 18.62 g/dl. Milking in this study was with 4 to 8 times stripping of the cord towards the baby till the cord is no longer distended 18.62[11].

Rabe at el conducted a randomized controlled trial on DCC versus UCM to increase the placental transfusion in preterm infants. Fifty-eight preterm infants were studied. DCC group had the cord clamped at 30s following delivery while the UCM group had the umbilical cord stripped 4 times at a 10cm/s speed from vulva towards the neonatal umbilicus. Both groups the infants were placed 20 cm below the level of the placenta at the mother's thighs. The mean Haemoglobin after 24 hours in the delayed cord

clamped group was 17.3g/dl and the mean haemoglobin of the milked group was 17.5mg/dl ($p = 0.71$)[13].

A study on UCM and ECC done in USA in 2008 with a sample of 24 showed that the milking group had a lower residual volume of blood in the placenta. The amount of blood in the placenta of the umbilical cord milked group was 13.2ml/kg compared to 19.2ml/kg in controlled group ($p<0.01$). Cord milked group had higher capillary haematocrit at 36-48hrs, amounting 57.5% compared to 50% in the early clamped group ($p<0.01$). There was no significant difference in serum bilirubin levels[12]. A similar study by Honso at el with 20 subjects for each arm showed no significant difference in haemodynamic states in the first 120hrs[19].

Upadhyay at el conducted a large randomized controlled study including 200 subjects. This study conducted in India in 2011, compared ECC with UCM of the term infants. The control group had the umbilical cord clamped within 30s of delivery while the intervention group had cord milking. The mechanism for milking they adopted as followed. The umbilical cord was clamped 25cm from the umbilical stump within 30s of delivery. Under the radiant warmer cord was milked 3 times at a speed of 10cm/s towards the umbilicus of the newborn and then was clamped leaving a length of 2 to 3cm. The haemoglobin levels at 12 hours of the control and intervention groups were 13.5g/dl and 15.1g/dl respectively. At 48 hours of birth these values were 10.8g/dl and 11.9g/dl. There was a significant difference between the control and intervention group haemoglobin levels at 12 hours and 48hours following delivery. Further they assessed the serum ferritin levels of these newborns at 6 weeks. The serum ferritin values were 177µg/l and 355µg/l in the early cord clamped and the cord milked group respectively. This also showed a significant difference. Only adverse effect noted with UCM was the significant increase in mean blood pressure[20].

Section – 3

Objectives

3.1 General objective

- To evaluate the effect on haemotological parameters of term neonates following umbilical cord milking compared to delayed cord clamping at elective caesarean section.

3.2 Specific objectives

- To compare the neonatal haemoglobin values and haematocrit in neonates undergoing cord milking and delayed cord clamping at caesarean section.
- To assess the haemodynamic changes and APGAR scores of neonates undergoing cord milking and delayed cord clamping at caesarean section.
- To compare serum bilirubin levels in neonates undergoing cord milking and delayed cord clamping at caesarean section.
- To assess the maternal blood loss in cord milking compared to delayed cord clamping.

Section – 4

Methodology

4.1 Study design

Randomized controlled trial

4.2 Study setting

Ward 5, Teaching Hospital Kandy

Operation Theatre C, Teaching Hospital Kandy

Haematology and Biochemistry Laboratories, Teaching Hospital Kandy

4.3 Study population

All pregnant mothers undergoing elective caesarean section at term in Kandy Hospital, from 1st February to 31st March 2015

4.4 Sampling

4.41 Sample size

According to a previous study done in 1950 comparing different techniques of umbilical cord clamping sample size was calculated. According to this study haemoglobin levels at 24 hours following delayed clamping and cord milking was 17.38mg/dl and 18.62mg/dl (SD – 1.5)[11]. The sample size thus calculated with 80% power at 5% significant level is 24 per each arm. [Sample size = $2*1.79/(18.62-17.38/1.5)^2 + 1$]

Expecting drop out rate of 15 to 20% due to blood sample collection from newborns the sample size was further inflated to 30 per each arm.

4.42 Randomization

A computer generated random number was given to consecutive mothers who were selected for elective caesarean section at term. The subjects receiving odd numbers were assigned for delayed cord milking and the subjects having

even numbers were assigned for cord milking. These numbers were only known by the midwife attending the caesarean section and were disclosed to the operator at the time of uterine incision. The mother, paediatric team who is doing the initial assessment and the laboratory technicians were blinded.

4.43 Inclusion criteria

All mothers undergoing elective caesarean section at 37 – 42 weeks of gestation.

4.44 Exclusion criteria

Umbilical cord less than 25cm
Major cord abnormalities (true knots)
Meconium stained liquor
Major Congenital anomalies of the fetus
Multiple pregnancies
Rh-negative mothers
Maternal diabetes
Oligohydramnios (AFI <5)
Estimated fetal weight < 2.5kg or >3.5kg
Antepartum haemorrhage or diagnosed placenta praevia or placental abruption

4.5 Method

On the admission, the admitting house officers selected mothers fulfilling the inclusion and exclusion criteria to the study. Selected mother were given the consent forms and explained about the study by the principal investigator. According to randomly generated numbers the antenatal ward midwife randomized these mothers in to the intervention and the control groups as documented earlier. Preoperative haemoglobin values of the participants were checked with drawing venous blood and the dates were rechecked going through the dating scans.

All the subjects underwent the caesarean sections under spinal anaesthesia at the Operating Theatre C of Teaching Hospital Kandy. The operating team was briefed on

the research and three surgeons who were trained on the two methods of umbilical cord clamping carried out all the surgical procedures.

Following delivery of the fetus all subjects were given intravenous oxytocin 5IU slowly according to the guidance on active management of the third stage of labour. The control group at this point had the umbilical cord clamped after 2 minutes or once the cord pulsations had ceased which ever was earlier. The intervention group had the umbilical cord milked towards the umbilicus of the baby three times with a 10cm/s speed per stroke followed by clamping of the cord 3 cm from the umbilicus. Rest of the surgery was carried out in the routine manner with two-layer closure of the uterus and meticulous achieving of haemostasis. Peritoneal defects were not sutured.

The newborns were immediately taken to the radiant warmer and the attending midwife and the peadiatric house officer assessed APGAR scores and neonatal heart rate at 5 minutes. Routine postoperative monitoring was carried out after the caesarean sections. All subjects had an intravenous infusion of 500ml of normal saline over six hours followed by early establishment of oral feeds.

Venous blood samples for maternal postoperative haemoglobin were withdrawn within 24 hours to 48 hours of the procedure. Venous blood samples were withdrawn from the newborns also during this period for assessment of venous haemoglobin, haematocrit and the serum total bilirubin values.

All the mothers were discharged within three to four days of delivery and were informed of the blood investigation results of themselves as well as their newborns. They were offered the routine follow up at 6 weeks at Teaching Hospital Kandy.

4.6 Outcome measures

4.61 Primary

- Venous haemoglobin and haematocrit of the newborns at 24 to 48 hours of birth

4.62 Secondary

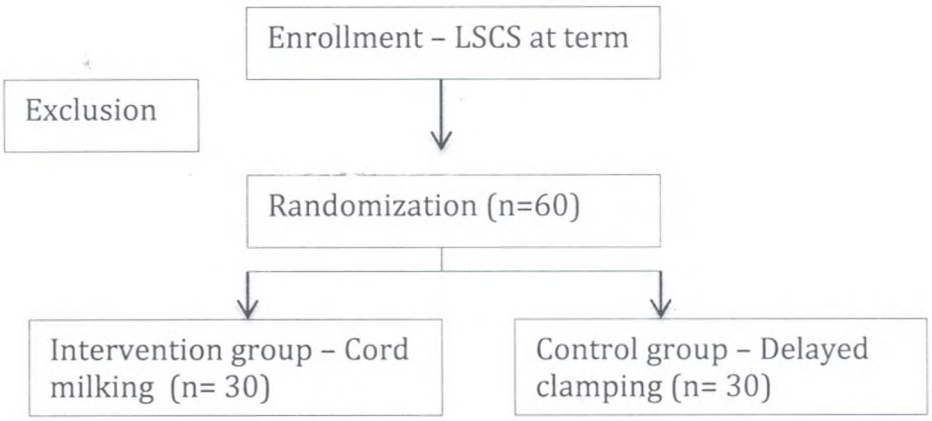
- 5 minute APGAR and heart rate of the neonate
- Serum bilirubin levels of neonate at 24 to 48 hours of birth
- Maternal haemoglobin values preoperative and 24 hours following LSCS

4.7 Instruments and measurements

Venous Haemoglobin and haematocrit values were assessed by the automated analyzer (coulter count®) at the haematology lab and the serum bilirubin levels were assessed by the automated analyzer at the biochemistry lab. These were quality controlled and calibrated daily.

4.8 Flow diagram

Figure 1 – Flow diagram for research



4.9 Analysis

Analysis was done using SPSS 22. Z test for difference in sample means were used to assess the differences in each outcome measures of continuous variables while the chi-squared test was used for the categorical data analysis.

Ethical considerations

All the participants were provided with written and verbal information on the study and informed written consent were taken. Any one of the subjects was allowed to withdraw from the study at anytime. Although the study was conducted in pregnant mothers the interventions were not life threatening or were not affecting the outcome of the pregnancy adversely. Mothers provided the consent for their neonates blood drawing.

On two occasions venous blood was drawn from the pregnant mothers for haemoglobin values. Trained staff carried this out. Our unit policy is to carry out routine preoperative and postoperative haemoglobin assessment for all caesarean sections. Therefore additional pricking of the mothers was not needed as a part of our study. The routine samples were used in our lab analyzer to assess the preoperative and postoperative haemoglobin values.

We had to perform an additional blood drawing, out of our routine practices to assess the neonatal venous haemoglobin, haematocrit and bilirubin levels. All these samples were collected at a single pricking by trained nurses. Before and after the procedure mothers' anxiety was addressed.

All samples were collected according to standard universal precautions with disposable finest available needles and utmost care to reduce the pain especially for the newborn during skin pricking.

Ethical approval for the study was taken from the Ethical Review committee of Teaching Hospital Kandy. Permission to carry out the research was granted by Director, Teaching Hospital Kandy.

Work plan

Figure 2 – Gantt chart

Activity	Month (5/14 to 8/15)								
	05	01	02	03	04	05	06	07	08
Writing the research proposal	X	X							
Ethical approval			X						
Sample recruitment and data collection				X	X	X	X		
Analysis								X	
Dissertation writing									X

Funding of the study

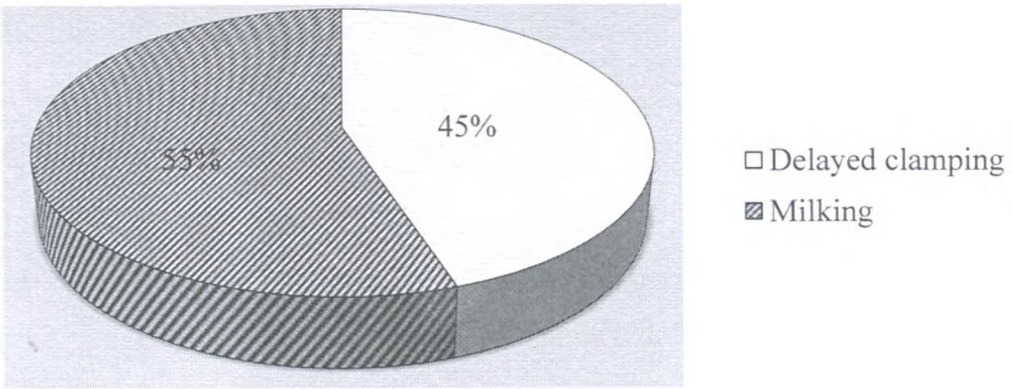
Funding will be arranged by the research fund of ward 5, Teaching Hospital Kandy.

Section – 5

Results

Hundred and twenty-eight pregnant mothers undergoing elective caesarean section were assessed for eligibility. Sixty subjects who fulfilled the inclusion criteria and gave consent were recruited for the study. Out of them 27 (45%) subjects underwent delayed cord clamping while 33 (55%) subjects underwent cord milking.

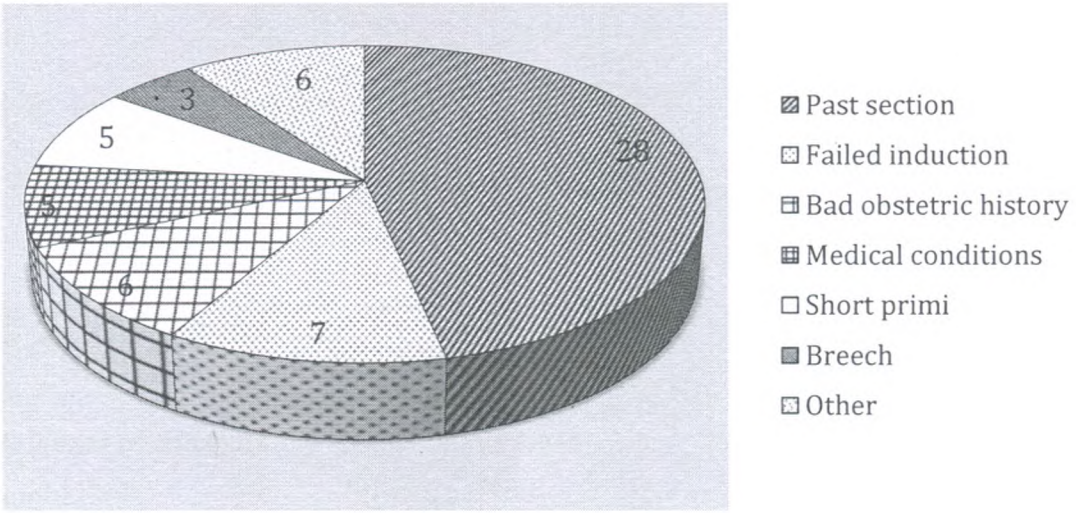
Figure 3 – Distribution of the sample



The period of amenorrhoea at the time of caesarean sections were from 38^{+0} to 40^{+6}. Main indications for the caesarean sections were past section (46.7%), failed induction (11.7%), past bad obstetric history (10.0%), Maternal medical conditions (8.3%), short primiparous mother (8.3%), breech (5.0%) and other (10%).

Mean maternal age of the delayed cord clamping group was 29.3 years and the milking group was 31.1 years. There was no significant difference between the pre-pregnancy maternal weights of the two groups.

Figure 4 – Distribution of the sample according to the indications for the caesarean section



There was no significant difference between the preoperative mean haemoglobin levels between the intervention and the control groups. All the pregnant mothers in the study were on iron and folic acid supplementation during the antenatal period. All had active management of the third stage, which include 5 units of slow intravenous oxytocin injection.

7 out of 44 had maternal medical disease in our sample. Using the chi-square with Fisher's extract test there was no significant difference between the two groups for occurrence of maternal medical diseases. The most frequent were maternal hypertensive disorders including chronic hypertension and pregnancy induced hypertension.

Mean birth weights of the neonates in the delayed cord clamping group was 2868.52 (min 2500, max 3450) and the cord milking group was 2988.79 (min 2500, max 3450). There was no significant difference between the birth weights of the newborns in the two study groups.

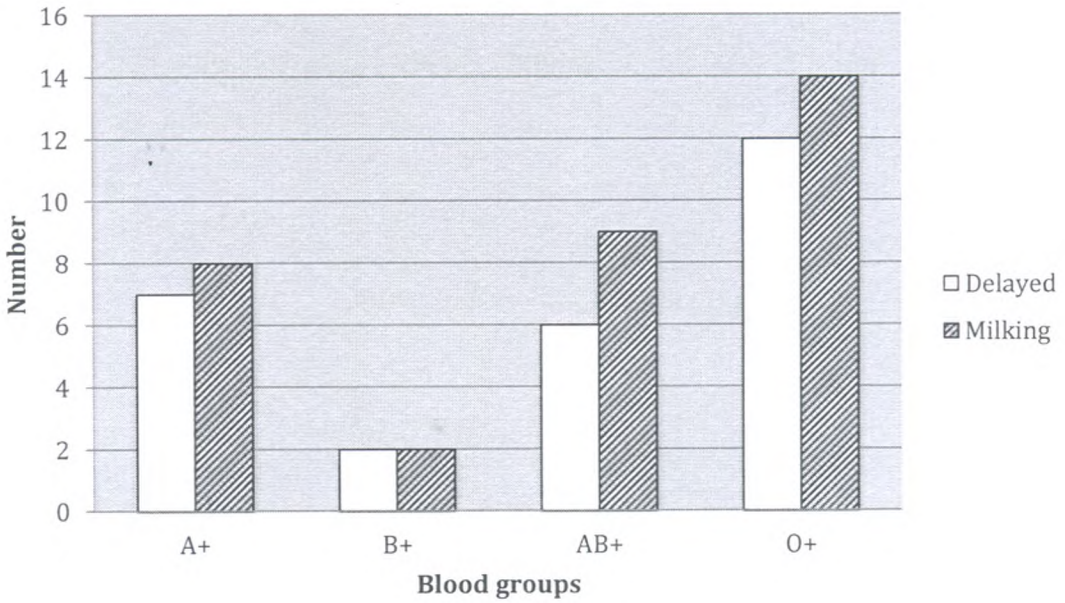
The sample characteristics are displayed in the below table. There was no significant difference between the control and the intervention groups for any of the assessed parameters.

Table 1 – Sample characteristics of the control and the intervention groups

	Delayed Cord clamping	**Cord Milking**	**P value**
Maternal age (years)	29.3 (±6.61)	31.1 (±4.63)	
Maternal pre-pregnancy weight (kg)	50.52 (±7.61)	51.42 (±8.14)	0.66
Maternal Hb (g/dl)	12.05 (±1.17)	11.99 (±1.059)	0.84
Use of oxytocin	100%	100%	NA
Antenatal iron supplementation	100%	100%	NA
Antenatal folic acid supplementation	100%	100%	NA
Medical conditions	3 (11.1%)	5 (15.2%)	0.72
Birth weight (g)	2869 (±233.35)	2989 (±295.37)	0.09
Placental weight (g)	543 (±105.61)	589 (±101.84)	0.10
Sex of new born (M)	12 (44.4%)	17 (51.5%)	0.57

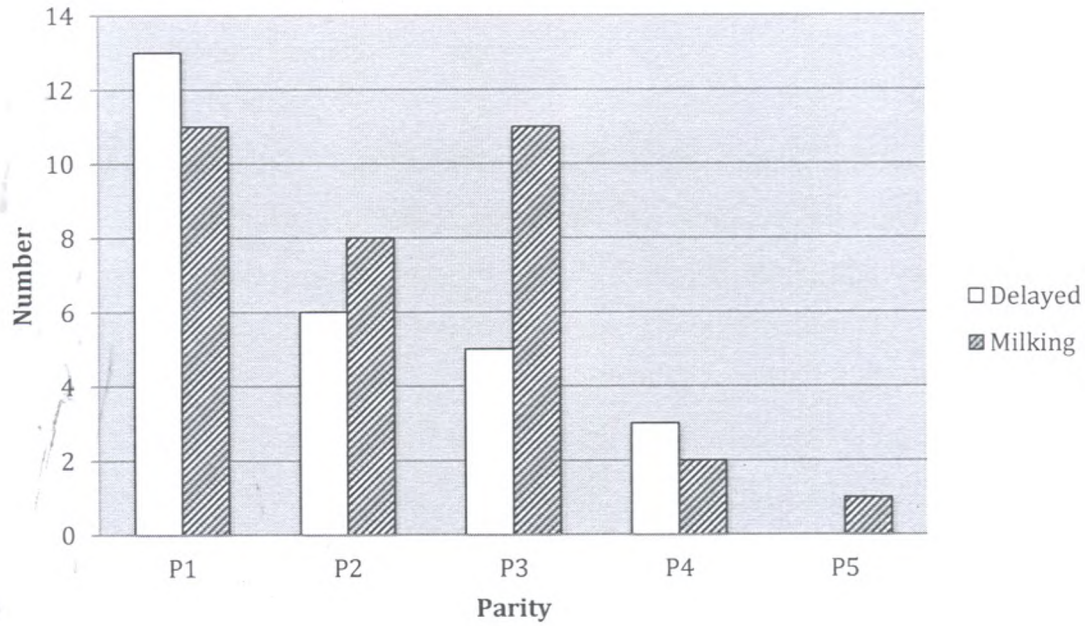
The commonest blood group in the sample was O+. There was no significance difference on distribution of the blood groups in the 2 arms of the research.

Figure 5 – Distribution of the study groups according to the blood groups



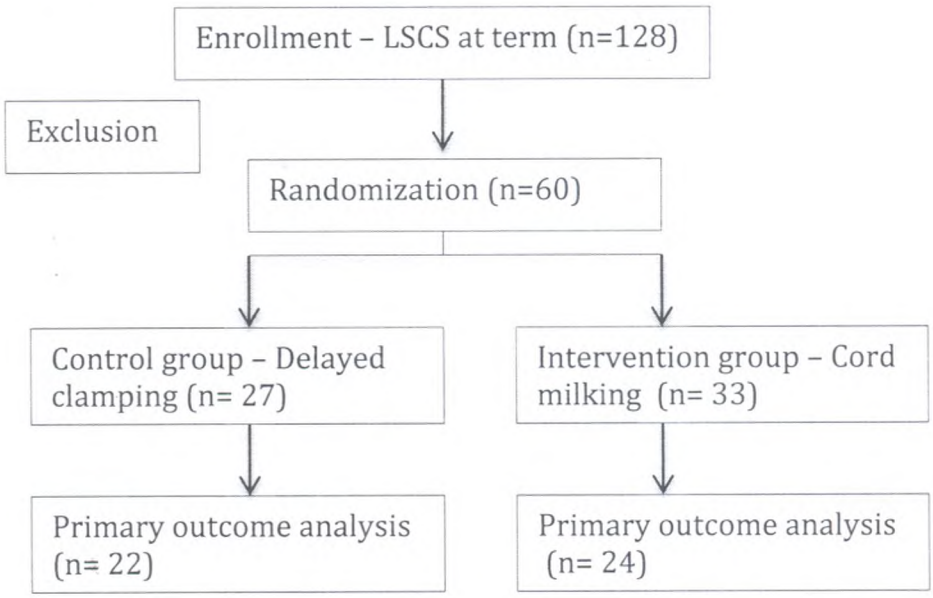
Most of the mothers in our study population were primiparous. The sample was equally distributed according to the parity without any significant difference between the control and the intervention groups. (x^2=0.50)

Figure 6 – Distribution of the study groups according to parity



Due to blood collection errors and mothers leaving the trial not giving consent for neonatal blood drawing only 47 samples were available for analysis of the primary outcome. This consisted of 22 (48%) samples of the control group and 24 (52%) samples of the intervention group.

Figure 7 – Trial profile



The analysis of the primary outcome of the study was the haemoglobin values and the haematocrit of the newborns.

The haemoglobin values of the newborns of both the control and the intervention groups were distributed normally. The mean haemoglobin concentration in the delayed cord clamping group was 17.364g/dl (±2.44) while the mean neonatal haemoglobin concentration in the intervention group was 17.642g/dl (±1.97).

Figure 8 – Histogram of neonatal haemoglobin distribution within first 24 hours in the delayed cord clamping group

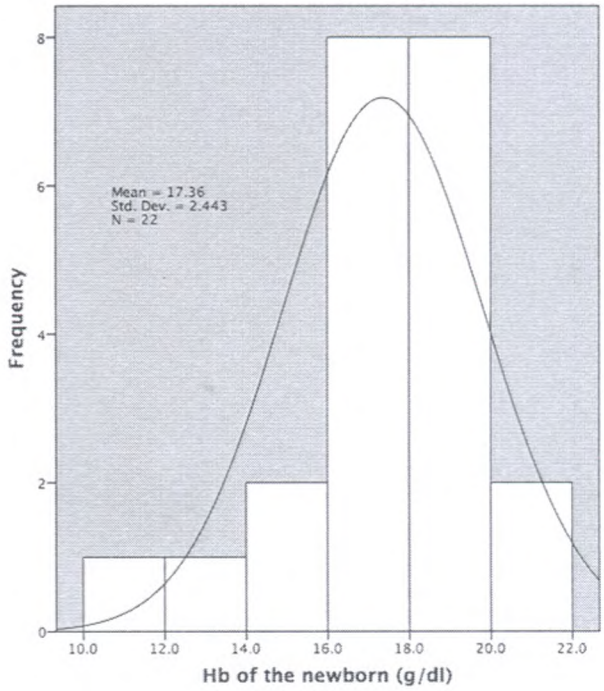
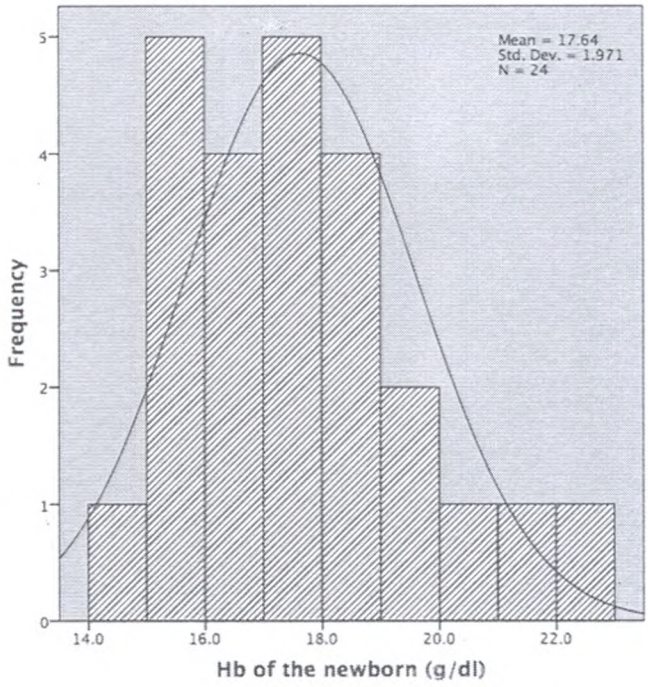
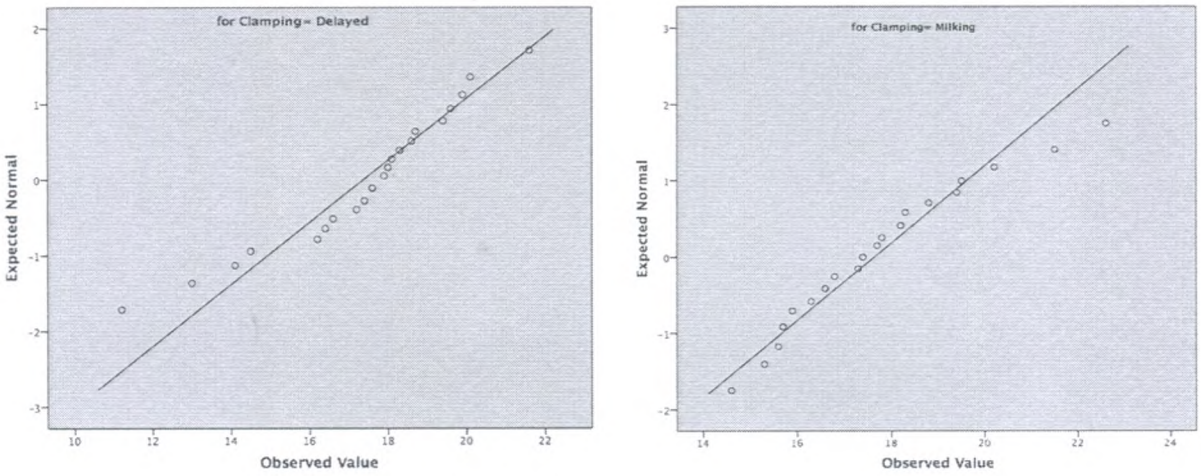


Figure 9 - Histogram of Neonatal haemoglobin distribution within first 24 hours in the cord milking group



The Q-Q plots for the two samples are shown below. Since data points were closed to the diagonal line we can further infer that the 2 data sets were normally distributed.

Figure 10 – Q-Q plots of the study groups



Further according to the test of normality for 2 samples (Shapiro wilk test) neonatal haemoglobin values in the two groups were normally distributed (Shapiro wilk = 0.24). There were no outliers in haemoglobin values of the newborns in the two groups. This is represented in the box and whisker plot below. The data sets were fulfilling all the criteria for application of independent sample t test, which we used for analysis of the primary outcome. Accordingly there was no statistical significance difference in haemoglobin values in 2 groups (p=0.67).

Figure 11 – Box and whisker plot of neonatal haemoglobin distribution with type of cord clamping

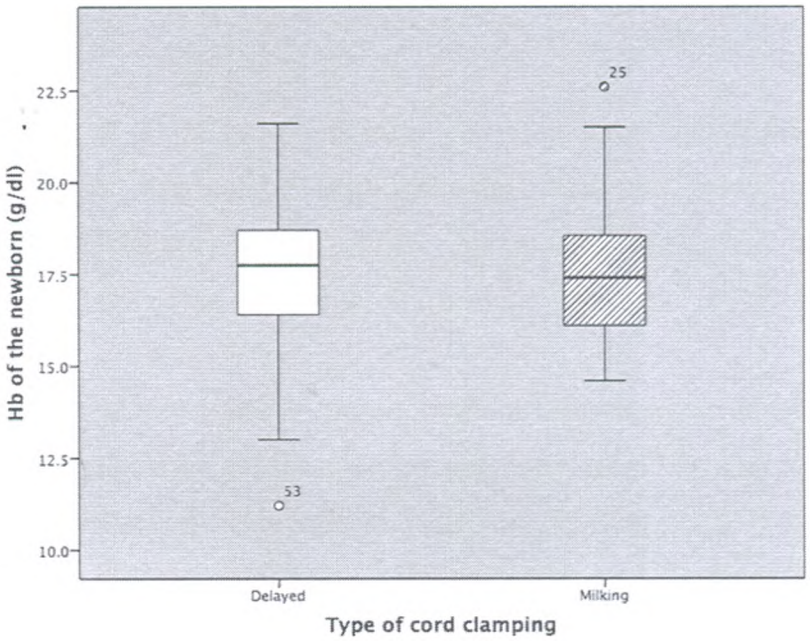
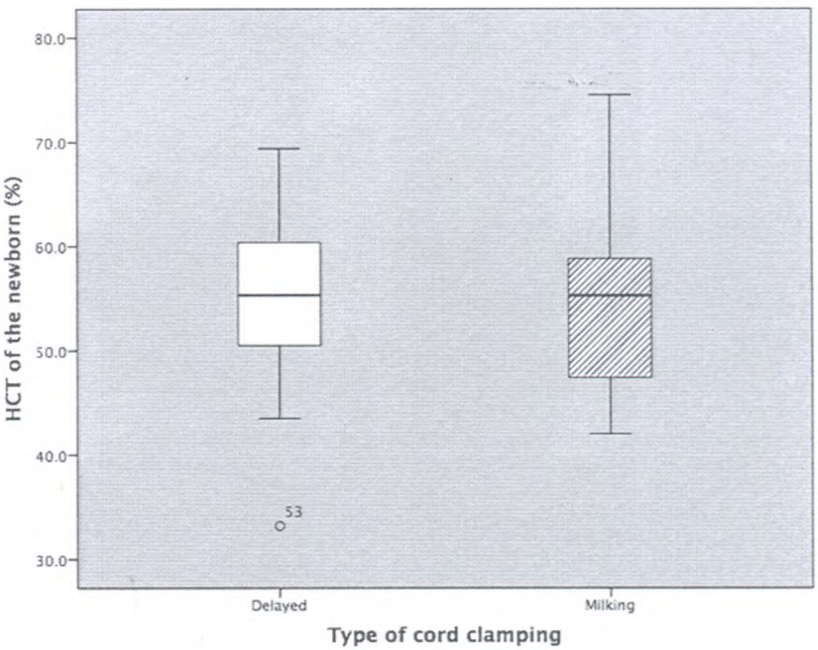


Figure 12 – Box and whisker plot of neonatal haematocrit distribution with type of cord clamping



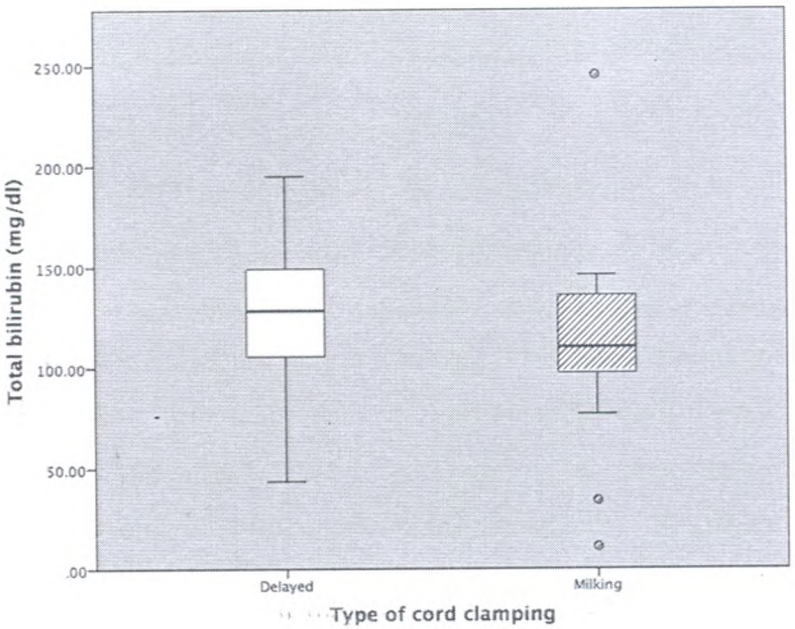
The haematocrit values of the newborns in two groups were analyzed similarly. The mean HCT of the delayed cord clamping group and the cord milking group were

54.90% (±8.46) and 54.47% (±7.48). There was no statistical significant difference between the two groups. The box and whisker plot for haematocrit of the newborns is shown above.

5 minute APGAR of all the newborns were ten. Mean heart rate of the neonates of the control group was 128 bpm (±15.50) while the intervention group neonates had a mean heart rate of 119 bpm (±5.26). There was no statistical significant difference between the heart rates of the two groups at 5 minutes after birth. (p=0.06)

When comparing the serum total bilirubin levels of these newborn at 24 hours to 48 hours from birth the delayed cord clamping group had a mean bilirubin value of 123.0 mmol/l (±34.99). The respective value for the cord milking group was 119 mmol/l (±5.26). Although the intervention group had a lower mean bilirubin value compared to the control group this did not reach the statistically significant level. (p=0.67). The box and whisker plot for the total serum bilirubin levels is shown below.

Figure 13 – Box and whisker plot of neonatal serum total bilirubin distribution in 24 to 48 hours from birth with type of cord clamping



The observations in the two study groups are summarized in the table below.

Table 2 – Observations in the study groups

	Delayed cord clamping	**Cord milking**	**p Value**
Haemoglobin of the newborn (g/dl)	17.364 (±2.44)	17.642 (±1.97)	0.67
Haematocrit of the newborn (%)	54.90 (±8.46)	54.47 (±7.48)	0.87
APGAR at 5minute (10)	100%	100%	NA
Neonatal HR at 5 minutes of birth (bpm)	128 (±15.50)	119 (±5.26)	0.06
Total bilirubin of the newborn (mmol/l)	123.0 (±34.99)	111.8 (±47.61)	0.67
Blood left in placenta	19.46 (±13.06)	29.45 (±20.25)	**0.03**
Hb drop (mg/dl)	1.27 (±0.95)	1.04 (±0.68)	0.37

We measured the reduction in the maternal haemoglobin value, from the preoperative to 24 hours following operations as an indirect assessment of blood loss at caesarean sections. The difference between the preoperative and the postoperative values were calculated to assess the blood loss in each subject. The mean haemoglobin drop after delayed cord clamping during caesarean sections was 1.27mg/dl (±0.95). The blood loss was lower when adhering to cord milking during caesarean sections accounting for only 1.04 mg/dl (±0.68). But this was not statistically significant according to independent t test for 2 sample means. (p=0.37)

Polycythemia is a known complication of delayed cord clamping. Polycythemia of the newborn is defined as the haematocrit value above 65%. Since the delayed cord clamping group had slightly higher haematocrit compared to the cord milking group we assessed the risk of developing polycythemia with different modalities of cord clamping.

Table 3 – Polycythemia with type of cord clamping

		Polycythemia	
		HCT ≥ 65	HCT < 65
Type of cord clamping	Delayed	15.0%	85.0%
	Milking	4.5%	95.5%

Odds ratio for polycythemia following delayed cord clamping was 3.75 (95% CI 0.35-38.93). Although the risk of a neonate to become polycythaemic was higher following delayed cord clamping compared to cord milking this difference was not significant.

Section – 6

Discussion

In our study we compared delayed cord clamping with cord milking. Although there were several studies comparing these interventions in preterm infants we could not find similar studies in term infants in the documented literature.

Upadyay at el conducted a similar study in 200 near term and term infants in India[20]. Although they were studying term infants, their comparison was between cord milking and early cord clamping as opposed to delayed cord clamping, which is the currently accepted method. According to this study there is a significant increment in haemoglobin values in neonates following cord milking. The mean haemoglobin values within 24 hrs of delivery in these infants were 13.4g/dl and 15.3g/dl in the early cord clamping group and the cord milking group respectively. However these values were well lower than our results for neonatal haemoglobin levels, which was 17.6g/dl and 17.4g/dl for the cord milking and delayed cord clamping groups respectively. The difference may be due to good maternal nutrition as well as universal iron and folic acid supplementation in our study sample leading to higher maternal iron stores compared to Indian pregnant mothers.

In a recent single center randomized trial by Rabe et al umbilical cord milking was compared with delayed cord clamping in preterm neonates taking 31 and 27 subjects in the two arms respectively[13]. This study was similar to our study with respect to the sample size except for the fact that the deliveries were preterm. They demonstrated comparable increase in haemoglobin in both the groups. The mean haemoglobin values were nearly similar to our study groups, and the values were 17.3g/dl for the delayed cord clamping group and 17.5g/dl for cord milking group.

Rabe et al milked the cord four times towards the baby where as we milked only three times[13]. In the delayed cord clamping group of their study they waited only for 30 seconds, which is below the current standards for delayed cord clamping. What we

practiced in our study was 2 minutes or till the cord pulsations ceased for the delayed cord clamping group. Upadhyay clamped the cord before milking[20]. This may be another reason for reduced haemoglobin values in their study groups compared to ours. We milked the cord before clamping like Rabe and Honso[19]. To increase the placental transfusion they employed another strategy of keeping the baby at the level of the mother. In our study design we did not concentrate on the effect of gravity since a meta-analysis by Palethorpe could not find evidence for the position of the baby with regards to the placental transfusion[21].

Reference ranges of neonatal bilirubin at 36 hours are 119 mmol/l for the 40th and 153 mmol/l for the 75th percentiles. In our study the cord milking group had mean serum bilirubin value of 111.8 ($\pm$47.61) mmol/l and the delayed clamping group had mean serum bilirubin of 123.0 ($\pm$34.99) mmol/l. There was no significant difference in serum bilirubin values in our study groups. In any of the studies conducted comparing cord milking with other methods of cord clamping could not demonstrate a significant difference in bilirubin values. This indirectly indicates that there is no increased risk of haemolysis following umbilical cord milking.

Katheria at el studied haemodynamic changes in preterm infants following immediate cord clamping compared to cord milking[22]. No difference was found in HR, BP although they found an increase in superior vena cava blood flows and systemic blood flows. They commented that theirs results were similar to a group of delayed cord clamped infants. (Meyer MP, Mildenhall L. Delayed cord clamping and blood flow in the superior vena cava in preterm infants: an observational study.). In our study there was no difference in heart rates within the two groups although we could not carryout more sophisticated measurements of blood flows.

APGAR scores were above 9 in all the infants even in preterm studies using various methods of cord clamping. So milking compared to delayed clamping does not make major changes with regards to the early acid base balances, which is indirectly represented by APGAR.

Cochrane review by McDonald at el showed that there is no significant difference in the risk of moderate to severe post partum haemorrhage by the timing of cord clamping (RR - 1.04, 95%CI 0.65-1.65) [6]. But there was a slightly increased risk of severe PPH with delayed cord clamping due to disturbing the normal process of active management of third stage of labour. Some of the studies in the review looked at maternal blood loss while other looked at maternal haemoglobin drop from preoperative to postoperative, which is an indirect measure of the blood loss. In our study the haemoglobin drop was 1.27g/dl for the delayed clamping group and 1.04g/dl for the cord milking group. The haemoglobin drops in our study were higher in comparison to the meta analysis findings. This difference may be explained by the fact that all our subject underwent caesarean sections with a higher blood loss compared to most undergoing vaginal deliveries in the Cochrane review. The difference between the two groups was not significant in our study. However there was a larger reduction of haemoglobin with delayed cord clamping, showing similarities to the meta analysis results.

Section – 7

Strengths and limitations

The main strength of our study was the meticulous way of cord milking, which was standardized and was carried out only by 3 surgeons who were trained on the exact method. We compared our method of cord milking with delayed cord clamping which is the currently recommended method. Having another arm with immediate cord clamping would have given more information to strengthen our intervention. However we did not include this arm in our study since early cord clamping compared to delayed cord clamping or milking has shown to have substandard increment in haemoglobin levels in most of the studies.

We used a sample size of 24 for each arm after calculations. Most of the studies, which were carried out in this field, had similar number of samples. Primary end point of the study was neonatal haemoglogin values. But there were problems associated with blood collection, which led to dropouts from the study. The problems encountered were the mothers changing their minds and not giving consent for blood collection of their newborns following caesarean section, collection errors such as unable to get an adequate sample as well as clotting of the samples during transport. These were the major limitations to our study with regards to the primary end point. Since we further inflated our sample by 20% expecting these dropouts we were able to overcome this problem without compromising on the quality of the research.

The ideal method for assessing the placental transfusion is the blood volume measurements. Blood volume assessment can be done with biotin labeling or red cells method, which is time consuming, expensive and difficult to perform on a large number of neonates. The other method is diluting adult hemoglobin in fetal blood and comparing this with circulating fetal hemoglobin, which is limited to neonates who require a donor blood transfusion. Because of these limitations we adhered to measurements in venous haemoglobin and haematocrit values. But if one of these expensive method had been used it would have given more clear information.

Our initial proposal was to assess the serum ferritin levels at 6 weeks as a method of checking iron stores. But the ethical committee did not justify summoning all research participants at 6 weeks and pricking the neonates additionally to collect these samples. Therefore we had to differ our proposal according to ethical committee requirements.

Therefore our study was only on short-term measurements. A long term follow up would have been more appropriate to check for long term effects of different methods of cord clamping. However the current evidence does not show significant long-term changes in outcomes when comparing delayed cord clamping to cord milking in preterm preterm infants.

Section – 8

Conclusions and recommendations

Umbilical cord milking achieves slightly higher neonatal haemoglobin values within 24 hours of birth without any additional risk of neonatal jaundice compared to delayed cord clamping. It also reduces maternal blood loss marginally during caesarean sections compared to delayed cord clamping. None of these positive findings achieved a significant difference. It can be safely concluded that umbilical cord milking is not an inferior procedure to delayed cord clamping.

Therefore umbilical cord milking can be recommended as an effective and a safe alternative to delayed cord clamping in term infants during caesarean deliveries.

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