RESEARCH PAPER

Effect of Delayed Cord Clamping on Hematocrit, and Thermal and Hemodynamic Stability in Preterm Neonates: *A Randomized Controlled Trial*

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Objective: To evaluate the short term clinical effects of delayed cord clamping in preterm neonates.

Design: Randomized controlled trial.

Setting: A tertiary care neonatal unit from October 2013 to September 2014.

Participants: 78 mothers with preterm labor between 27 to $31^{6/7}$ weeks gestation.

Intervention: Early cord clamping (10 s), delayed cord clamping (60 s) or delayed cord clamping (60 s) along with intramuscular ergometrine (500 µg) administered to the mother.

Main outcome measures: *Primary:* hematocrit at 4 h after birth; *Secondary:* temperature on admission in neonatal intensive care unit, blood pressure (non-invasive) at 12 h, and urinary output for initial 72 h.

Results: Mean (SD) hematocrit at 4 h of birth was 58.9 (2.4)% in delayed cord clamping group, and 58.7 (2.1)% in delayed cord clamping with ergometrine group as compared to 47.6 (1.3)% in early cord clamping group. Mean (SD) temperature on admission in NICU was 35.8 (0.2)ºC, 35.8 (0.3)ºC, and 35.5 (0.3)ºC, respectively in these three groups. The mean (SD) non-invasive blood pressure at 12 h of birth was 45.8 (7.0) mmHg, 45.8 (9.0) mmHg, and 35.5 (8.6) mmHg, respectively in these three groups. Mean (SD) urinary output on day 1 of life was 1.1 (0.2) mL/kg/h, 1.1 (0.2) mL/kg/hr and 0.9 (0.2) ml/kg/h, respectively.

Conclusion: In preterm neonates delayed cord clamping along with lowering the infant below perineum or incision site and administration of ergometrine to mother has significant benefits in terms of increase in hematocrit, higher temperature on admission, and higher blood pressure and urinary output during perinatal transition.

Keywords: *Anemia, Hypothermia, Newborn resuscitation, Umbilical cord*.

Optimal cord clamping time in premature neonates remains controversial [1]. Delayed cord clamping (DCC) is considered a more physiological mechanism by which the newborn may receive an additional blood volume to perfuse the lungs, intestines, kidneys and the skin, favoring successful adaptation to the extrauterine life [2], and has been recently recommended as preferred method of cord clamping in preterm neonates, when feasible [3]. However, other birth-related factors also influence the speed and amount of placental transfusion at birth; the level at which the infant is held; the type and method of delivery; uterine contractions during third stage; and administration of uterotonics (oxytocin or ergometrine) [4]. Yao, *et al*. [5] demonstrated that a strong uterotonic drug administered intravenously to the mother increased the rate of placental transfusion without a risk of over transfusion. We conducted this single center randomized controlled trial to test the primary hypothesis that for preterm neonates, lowering the infant below the perineum/incision site, administration of intramuscular ergometrine to the mother and delayed cord clamping will result in higher hematocrit and improved thermal and hemodynamic stability.

METHODS

The study was conducted over a period of 12 months (October 2012 to September 2013) at a tertiary care hospital in Mumbai, India. The study was approved by institutional ethics committee of the hospital. Mothers with $27\text{-}31^{6/7}$ weeks' gestation with preterm onset of labor were included to participate in the study. Mothers with multiple gestation, Rh-ve status, placenta previa or abruption-placenta, and those having fetus with major congenital anomalies, hydrops, fetal growth restriction with abnormal Doppler waveforms, or evidence of foetal distress were excluded from the study. Mothers, who fulfilled the inclusion criteria, when they came in labor, were enrolled in the study during onset of labor after obtaining informed consent. Mothers were assigned to

Early cord clamping (ECC) group, Delayed cord clamping group 1(DCC1) or Delayed cord clamping group 2E (DCC 2E). For the ECC group, obstetrician clamped the umbilical cord at 10 seconds and baby was held supine at level of introitus/placental incision. For the DCC1 group, neonates were held in a pre-warmed towel approximately 10-15 inches below the introitus at vaginal delivery/below the level of placental incision in caesarean delivery, and cord was clamped at 60 seconds. For the DCC2E group, neonates were held 10-15 inches below the introitus at vaginal delivery/below the level of placental incision in caesarean delivery, injection ergometrine 500 µg intramuscular (IM) was administered to the mother, and cord was clamped at 60 seconds. A stopwatch was used to mark the time that was counted in 10 seconds interval. Allocation of groups was done by random number sequence with variable block size of 3 or 6 using a 'Random Allocation Software' program. The sequence was concealed in serially numbered, opaque, sealed and identical envelopes. The random allocation sequence was generated by a statistician who was not a part of the study.

Antenatal and delivery details were entered in mother's chart. Umbilical cord blood was collected for blood gas analysis within 30 minutes of collection. Timing of cord clamping, APGAR score at 1 min and 5 min, and time of birth were recorded. Axillary temperature was recorded with a mercury thermometer in labor room at approximately 5 minutes.

After stabilization in labor room, neonates were shifted to neonatal intensive care unit (NICU) and managed as per standard protocol. At 4 hours of age, venous sample was collected for hematocrit measurement. At 12 hour, heart rate, mean non-invasive BP (NIBP), Clinical Risk Index for Babies (CRIB) score, max FiO_2 requirement, respiratory support during initial 24 hours, requirement of surfactant, and arterial/alveolar ratio at 24 hour were recorded in all neonates. For initial 72 hours, urinary output was recorded. At 7, 14 days and 40 weeks post-menstrual age, neurosonogram was performed. Neonates were followed-up for retinopathy of prematurity (ROP) screening and subsequent retinal examinations.

Primary outcome was hematocrit at 4 hours of age. Secondary outcomes were temperature on admission, heart rate, NIBP at 12 hours, urinary output for initial 72 hours, number of red cell transfusions, total serum bilirubin (TSB) at 72 hours, peak serum bilirubin (PSB), evidence of retinopathy of prematurity (ROP), intra-ventricular hemorrhage (IVH), late onset sepsis (LOS), and nectrozing enterocolitis (NEC) stage 2 or more.

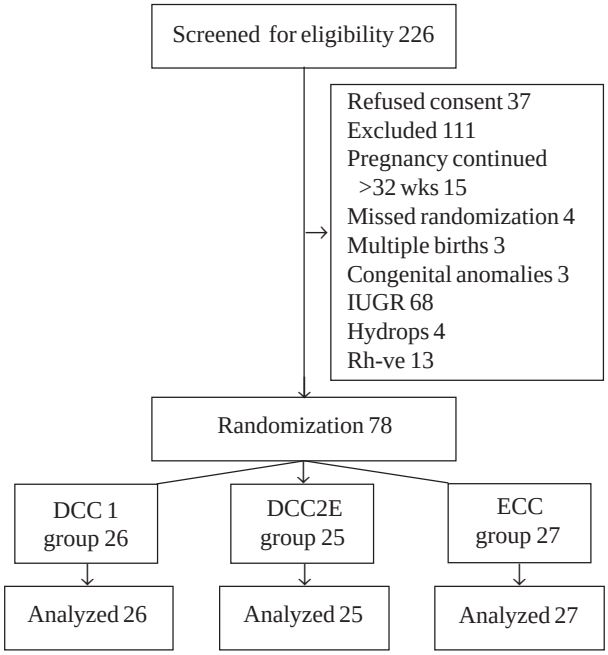
Sample size calculation was based on venous hematocrit at 4 hours of age in immediately clamped infants. Considering it as [48(4) % SD] [5,6] and using initial venous hematocrit as the primary outcome variable and an expected 10 to 15% relative increase by DCC with an alpha error 0.05 and power 80%, we estimated the need to enrol 30 neonates in each group. Statistical analysis was performed using SPSS version 16. Data were analyzed by intention to treat principle. $P<0.05$ was considered as statistically significant.

RESULTS

Flow of recruitment of study participants is shown in (***Fig.* 1**).The demographic profile was comparable in all the three groups (***Table* I)**.

Mean (SD) venous hematocrit at 4 hours was 47.6 (1.3)% in ECC group as compared to 58.9 (2.5)% in DCC1 and 58.7 (2.1)% in DCC2E group. There was statistically significant mean (SD) difference in venous hematocrit in DCC1 group when compared with ECC group, and when DCC2E was compared with ECC group (***Table* II**). In ECC group, 6 infants required vasopressor support, while in DCC1 group and DCC2E, 1 and 2 infants, respectively required vasopressor support.

Among three groups, 11 (40.7%), 2 (7.7%), 1 (4%) in ECC, DCC1 and DCC2E groups, respectively required red cells transfusion. Mean (SD) TSB at 72 hours was 9.4



ECC: early cord clamping; DCC1: delayed cord clamping; DCC2E: delayed cord clamping with intramuscular ergometrine.

FIG. 1 *Flow of recruitment of study participants.*

TABLE I COMPARISON OF BASELINE CHARACTERISTICS IN THE THREE STUDY GROUPS

Maternal and infant characteristics	*ECC(n=27)*	*DCC1(n=26)*	*DCC2E(n=25)*
Mother's age (y)	26.6 (4.2)	26.6 (3.9)	26.0 (4.2)
*Gestational age (wk)	29.9 (1.4)	30.1(1.2)	30.2 (1.2)
Caesarian delivery	4 (14.8)	4 (15.4)	4 (16)
Chorioamnionitis	3 (11.1)	3 (11.5)	2 (8)
Birth Weight (g)	1283.7 (176.4)	1316.1 (162.9)	1297.9 (177.8)
*Baseline Temperature at 5 min after birth (^{o}C)	34.0 (0.7)	33.9 (0.8)	34.2 (0.5)
*Resuscitation required	7 (26%)	8 (31%)	6 (24%)
*Cord blood pH	7.2 (0.1)	7.2 (0.1)	7.2 (0.1)

*ECC: early cord clamping; DCC1: delayed cord clamping; DCC2E: delayed cord clamping with intramuscular ergometrine. Values in n (%) or *mean (SD).*

TABLE II COMPARISON OF OUTCOME MEASURES BETWEEN THE THREE GROUPS

Characteristics	*Post Hoc multiple comparison ,weighted mean difference (SD) between groups ECC and DCC1(P value)*	*Post hoc multiple comparison, weighted mean difference (SD) between groups ECC and DCC2E (P value)*
Venous hematocrit at 4 hours	11.3 (0.6) (<0.001)	11.1 (0.6) (<0.001)
Admission temperature	0.3 (0.1) (0.004)	0.3 (0.1) (0.008)
Mean BP at 12 hours (mm hg)	10.2 (2.3) (<0.001)	10.3 (2.3) (<0.001)
Urine Output in first 24 hours (ml/kg/hr)	0.3 (0.1) (<0.001)	0.2(0.1) (<0.001)
Urine Output in next 24 hours (ml/kg/hr)	0.7 (0.1) (<0.001)	0.7 (0.1) (<0.001)
TSB at 72 hours (mg/dL)	3.8 (0.8) (<0.001)	3.8 (0.8) (<0.001)
PSB (mg/dL)	2.1 (0.9) (0.02)	2.5 (0.9) (0.01)

**TSB: Total serum bilirubin , # PSB : Peak serum bilirubin; ECC: early cord clamping; DCC1: delayed cord clamping; DCC2E: delayed cord clamping with intramuscular ergometrine.*

(3.1) mg/dL in DCC1 and 9.4 (3.3) mg/dL in DCC2E as compared to 5.6 (1.7) mg/dL in ECC group. In ECC group, 10/27 (37%) had evidence of brain injury while 6/26 (23.1%) in DCC1 group and 5/25 (20%) in DCC2E had evidence of brain injury. None of the neonates in DCC group required therapy for ROP while 2 (7.4%) babies in ECC group underwent laser phototherapy. Nine (33.3%) babies in ECC group, 5 (19.2%) in DCC1 and 3 (12%) babies in DCC2E group developed late onset sepsis. Evidence of hemodynamically significant patent ductus arteriosus (hsPDA) was seen in 5 (18.5%), 4 (15.4%) and 2 (8%) neonates in ECC, DCC1 and DCC2E groups, respectively.

There was no significant difference among three groups for maximum FiO_2 requirement in first 12 hours, heart rate at 12 hours, respiratory support needed, surfactant administration, CRIB score in first 12 hours, a/A ratio, arterial blood pH, base deficit at 12 hours, LOS during NICU stay, evidence of brain injury on USG skull, ROP needing treatment or presence of hsPDA requiring medical closure.

DISCUSSION

In this randomized controlled trial, higher venous hematocrit values at 4 h of age in delayed clamping groups indicate that delayed cord clamping is effective in increasing the placental transfusion in preterm neonates. Infants in delayed clamping group had improved temperature on admission in NICU in our study but there was no difference between DCC1 and DCC2E group.

One of the limitations of this study was that we did not measure effects of delayed cord clamping on blood volume. However, we checked the indirect manifestations of increased blood volume such as initial hematocrit, blood pressure and urinary output. Recording of only short-term effects of delayed clamping could be another limitation. Outcomes of delayed cord clamping were not studied in cases of growth retarded babies and in

WHAT IS ALREADY KNOWN?

- Delayed cord clamping in preterm neonates is associated with improved hematocrit and less incidence of anemia at 6-10 weeks of age.

WHAT THIS STUDY ADDS?

- This study demonstrates the cumulative effects of (*i*) lowering the infant position by 10-15 cm below the perineum/ incision site; (*ii*) administration of inj ergometrine; in addition to (*iii*) delayed cord clamping on placental transfusion.
- This better placental transfusion, is associated with less hypothermia on admission in NICU and improved blood pressure and urinary output during the perinatal transition.

non-vigorous neonates requiring resuscitation. Apart from timing of cord clamping, position of infants and use of ergometrine after delivery, other factors which affect the placental transfusion *i.e.* mode of delivery (vaginal versus caesarean), were not compared. Single-center based study and small sample size were other limitations.

Finding of raised hematocrit is consistent with observations of Ibrahim, *et al*. [8], who reported that even 20 seconds' delay in cord clamping could cause rise in hematocrit at 4 hours. Oh, *et al.* [9] also documented that a delay of 30-45 seconds caused rise of hematocrit. However, Mc Donnel, *et al*. [10] found no difference in hematocrit at 4 hours in relation to timing of cord clamping. A Cochrane systemic review [7] concluded that DCC is associated with fewer transfusion requirements for anemia; for every 100 babies subjected to delayed cord clamping, 27 are spared a blood transfusion. Further studies with large sample size are required to evaluate whether delayed cord clamping in preterm neonates leads to low incidence of complications such as late onset sepsis, necrotizing enterocolitis, bronchopulmonary dysplasia or retinopathy of prematurity. Studies can be undertaken for considering the possibilities of delayed clamping in preterm neonates who require immediate resuscitation so that both resuscitation and delayed cord clamping can occur simultaneously. We conclude that in preterm infants, the placental transfusion achieved with lowering the infant below perineum or incision site, administration of intramuscular ergometrine and delayed cord clamping by 60 seconds, is associated with less hypothermia on admission in NICU and improved blood pressure and increased urinary output during the perinatal transition.

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