RESEARCH PAPER

Effect of Umbilical Cord Milking *vs* Delayed Cord Clamping on Venous Hematocrit at 48 Hours in Late Preterm and Term Neonates: *A Randomized Controlled Trial*

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Objective: To compare the effect of intact umbilical cord milking (MUC) and delayed cord clamping (DCC) on venous hematocrit at 48 (±6) hours in late preterm and term neonates ($35^{0/7}$- $42^{6/7}$ wk).

Study Design: Randomized trial.

Setting and participants: All late preterm and term neonates ($35^{0/7}$ - $42^{6/7}$ wk) neonates born in the labor room and maternity operation theatre of tertiary care unit were included.

Intervention: We randomly allocated enrolled neonates to MUC group (cord milked four times towards the baby while being attached to the placenta; *n*=72) or DCC group (cord clamped after 60 seconds; *n*=72).

Outcome: Primary outcome was venous hematocrit at 48 (±6) hours of life. Additional outcomes were venous hematocrit at 48 (±6) hours in newborns delivered through lower segment caesarean section (LSCS), incidence of polycythemia requiring partial exchange transfusion, incidence of hyperbilirubinemia requiring phototherapy, and venous hematocrit and serum ferritin levels at 6 (±1) weeks of age.

Results: The mean (SD) hematocrit at 48 (±6) hours in the MUC group was higher than in DCC group [57.7 (4.3) *vs.* 55.9 (4.4); *P*=0.002]. Venous hematocrit at 6 (±1) weeks was higher in MUC than in DCC group [mean (SD), 37.7 (4.3) *vs.* 36 (3.4); mean difference 1.75 (95% CI 0.53 to 2.9); *P*=0.005]. Other parameters were similar in the two groups.

Conclusion: MUC leads to a higher venous hematocrit at 48 (±6) hours in late preterm and term neonates when compared with DCC.

Keywords: *Anemia, Infant, Placental redistribution, Transfusion,*

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Placental transfusion provides sufficient iron reserves for the first 3 to 6 months of life; thus, preventing or delaying the development of iron deficiency until the use of iron-fortified foods is implemented [4]. Delayed cord clamping (defined variably as clamping till cessation of pulsations or up to 60-180 seconds) leads to improvement in levels of hemoglobin and hematocrit at two months of age [5]. However, universal application is limited in particular due to obstetrician concerns for the risk of hypothermia, and delay in initiation of resuscitation, when indicated [6,7].

Umbilical cord milking, on the other hand, involves milking the entire contents of the umbilical cord towards the baby. Cut umbilical cord milking (umbilical cord detached from placenta) limits the refilling of cord from placenta, so less blood is likely to be transfused when compared to intact umbilical cord milking (pushing the blood toward the infant at least four times before clamping the umbilical cord) [8-12].

Till date, two studies [13,14] have evaluated the effect of delayed cord clamping and umbilical cord milking in term neonates. In both studies, cut umbilical cord milking was performed. Hence, we planned the present study to evaluate the effect of intact umbilical cord milking on venous hematocrit at 48 hours of age in late preterm and term neonates when compared with delayed cord clamping.

METHODS

This open labelled randomized trial was conducted in the department of obstetrics and gynecology, All India Institute of Medical Sciences, New Delhi from May to September, 2016. All late preterm and term neonates ($35^{0/7}$ - $42^{6/7}$ week) were included in the study. Neonates with fetal hydrops, major congenital malformation, Rh isoimmunization (Rh positive neonate born to Rh negative mother with indirect Coombs test (ICT) positive) [15], newborns born through meconium stained

liquor who were non-vigorous at birth (defined by poor/no respiratory efforts, weak/no muscle tone and heart rate less than 100 beats per minute, limp or apneic or poor tone at birth) [16], forceps or vacuum assisted delivery, and newborns born to HIV positive mother (on enzyme-linked immunosorbent assay (ELISA) followed by Western blot test for HIV) and maternal eclampsia (defined as generalized seizure in pregnant females with preeclampsia) [17] were excluded from the study. The study was approved by the institutional ethics committee.

All eligible mothers admitted in the labor room were screened for eligibility and enrolled, after informed written consent. We allocated mothers using computer generated random sequence to intact umbilical cord milking group and delayed cord clamping group. Opaque envelopes containing allocation group were serially numbered, and sealed to conceal the identity. The sealed envelope was opened by the nursing staff when the expectant mother was wheeled inside the labor room. The intervention written on slip was carried out by the obstetrics and gynecology resident and pediatric resident team posted in the labor room. Blinding of the clinicians was not possible due to the obvious nature of intervention.

Intact umbilical cord milking (MUC) group: The intact umbilical cord for its remaining accessible length (nearly half of the total length) was milked four times towards the baby by the residents on duty in the obstetrics department, and then clamped. All the health care providers (postgraduate residents of pediatrics and obstetrics) were trained in a structured manner for intact umbilical cord milking.

Delayed cord clamping (DCC) group: Umbilical cord was clamped at least 60 seconds from the time of delivery.

Time of all interventions was recorded by a stopwatch and noted in the study form. In both the groups, the baby was held at introitus after vaginal delivery, and over mother's thigh in caesarean delivery. After delivery, the babies were kept with mothers unless they required admission in the neonatal intensive care unit (NICU) for standard indications. Gestational age was assigned based on the last menstrual period. The appropriateness of birthweight for gestational age was assigned by stan-dard intrauterine growth chart [18]; weight less than 10th centile and weight more than 90th centile being adjudged as small for gestational age (SGA) and large for gestational age (LGA), respectively [18]. Early breastfeeding was encouraged in all babies as per standard guidelines. The infants were evaluated at birth, and at the age of 24 hours and then at 48 hours.

The primary outcome was venous hematocrit evaluated at 48 (±6) hours. Additional outcomes were venous hematocrit at 48 (±6) hours in newborns delivered by lower segment caesarean section (LSCS), incidence of polycythemia (defined as venous hematocrit greater than 65% at 48 (±6) hours of life) [19] requiring partial exchange (PET), incidence of hyperbilirubinemia requiring phototherapy (as per American Academy of Pediatrics (AAP) charts) [20], venous hematocrit at 6 (±1) weeks, and levels of serum ferritin at 6 (±1) weeks.

Venous sample was collected in micro- capillaries for measurement of hematocrit, and an additional 1 mL sample was separately collected for serum ferritin levels. Micro-capillaries were micro-centrifuged at a speed of 9000 rpm for 5 minutes and analyzed with card reader for hematocrit measurement. Serum bilirubin was assessed in babies with clinical icterus by spectrophotometer (Apel BR 5100, APEL). Calibration of the spectrophotometer was done at defined intervals, as recommended by the manufacturer and phototherapy was instituted, if required. Serum ferritin levels were evaluated with ELISA orgentech kit (analytical sensitivity, 5 ng/mL; range of evaluated concentrations 5-1000 ng/mL).

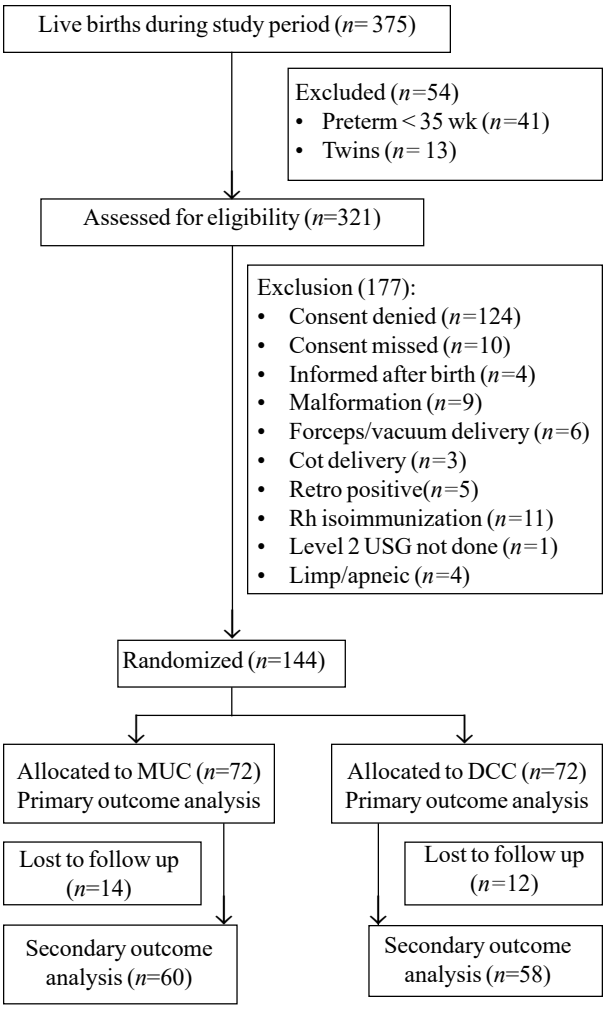
Attendants were counseled by the principal investigator to follow up at 6 (±1) weeks, which coincided with their immunization visit. Hematocrit evaluation and serum ferritin levels were evaluated at this time point. On follow up, parents were asked about any intercurrent illnesses since birth, and type and mode of feeding (top fed, exclusively breastfed or predominantly breastfed.

Venous hematocrit at 48 (±6) hours in a previous study was 50% [10]. Anticipating that intact umbilical cord milking will lead to at least a 5% absolute increase in the hematocrit and assuming a standard deviation (SD) of 7 in each group with power of 90% and alpha of 0.05, we needed to enroll at least 42 neonates in each arm. Considering an attrition rate of 40% on follow up, total sample size was increased to 72 in each group.

Statistical analyses: Statistical analyses were performed with Stata 11 (Stata Corp LP). Baseline categorical variables were compared using Chi-square or Fisher exact test, as appropriate, and whereas continuous variables were compared using Student t-test. A P-value of less than 0.05 was considered as significant. The analysis was by the intention to treat.

RESULTS

A total of 375 babies were delivered during the enrolment period, of which 144 babies fulfilled the inclusion criteria and were enrolled (***Fig.* 1**). Baseline characteristics including maternal pregnancy induced hypertension,



MUC: Milking of umbilical cord, DCC: Delayed cord clamping.

Fig. 1 *Study flow chart.*

gestational diabetes, gestational age and birthweight were comparable between the two groups (***Table* I**). 72 neonates were enrolled to DCC and 72 neonates to MUC group. Out of the 144 neonates, 118 (82%) completed the trial at 6 (±1) weeks. There were no adverse events in either group during the study period.

The mean (SD) hematocrit at 48 (±6) hours in the MUC group [57.7 (4.3)] was significantly higher than the DCC group [55.9 (4.4)] [mean difference (MD) 1.7 (95% CI 0.21 to 3.1); *P*=0.002] (***Table* II**). Venous hematocrit in newborn delivered by caesarean section at 48 (±6) hours was similar in the two groups. Incidence of polycythemia was also similar in the two groups. One neonate in each group required phototherapy. Mean (SD) Venous hematocrit at 6 (±1) weeks was higher in MUC than in DCC group [MD (95% CI) 1.75 (0.53 to 2.9); *P*= 0.005] (***Table* II**). The levels of serum ferritin were similar in the two groups (***Table* II** and ***Fig.* 2**).

Table I Baseline Maternal and Neonatal Characteristics of the Two Groups

	Umbilical cord milking group (n=72)	*Delayed cord clamping group (n=72)*
Maternal characteristics		
Booked pregnancy	72 (100)	71 (99)
Maternal age (y)*	29.1 (4.2)	28.3 (3.3)
Lower segment caesarean section	31 (43)	36 (50)
Hemoglobin (g/dL)*	11.7 (1.2)	11.4 (1.1)
Chronic hypertension	7 (9.7)	4 (5.5)
Pregnancy induced hypertension	3 (4.1)	3 (4.1)
Meconium stained liquor	2 (2.7)	6 (8.3)
Intra uterine growth retardation	1 (1.4)	3 (4.1)
Gestational diabetes mellitus	13 (18.1)	11 (15.2)
Neonatal characteristics		
Gestation age (wk)*	37.9 (1.0)	37.8 (1.6)
Male sex	36 (50)	36 (50)
Small for date	1 (1.4)	0
Large for date	10 (13.9)	9 (12.5)
Weight (g)*	3038 (436)	2909 (435)
Use of any respiratory support	3 (4.1)	3 (4.1)
Admission in NICU	1 (1.4)	2 (2.8)
Time since cord clamp (s)*#	12.9 (0.8)	60 (0)

*Data depicted as n (%) or *mean (SD); All P<0.05 except #P<0.01.*

DISCUSSION

This randomized trial compared intact umbilical cord milking (MUC) with delayed cord clamping (DCC) on venous hematocrit at 48 (±6) hours of life in late preterm and term neonates. The hematocrit at 48 (±6) hours and at 6 (±1) week was higher in the intact MUC group. However, it was similar in the two groups in infants delivered by LSCS. Other parameters including incidence of polycythemia, incidence of hyperbilirubinemia requiring phototherapy and ferritin were similar in the two groups.

There are very few studies in late preterm and term infants comparing MUC with DCC. Jaiswal, *et al*. [13] evaluated the effect of MUC and DCC on hematological parameters (serum ferritin and hemoglobin) at 6 (±1) weeks of life in term neonates. The packed cell volume (PCV) at 48 (±6) hours and hemoglobin level at 6 (±1) weeks postnatal age was similar in the two groups in contrast to the results of the present study. Studies in preterm infants comparing DCC suggest mixed results [8,9,11,12]. The cord vein contains nearly 20 mL of placental blood and one-time umbilical cord milking (of

Table II Primary and Secondary Outcome Variables in Late Preterm and Term Neonates in the Study

Parameter	*MUC group (n=72)*	*DCC group (n=72)*	*Mean difference (95% CI)*
Hematocrit at 48 (±6) h	57.7 (4.3)	55.9 (4.4)	1.68 (0.21, 3.1)
Secondary outcomes	(*n* = 58)	(*n* = 60)	
Hematocrit[#]	37.7 (3.3)	36 9 (3.4)	1.7 (0.53, 2.9)
Serum ferritin (ng/mL)[#]	363.1	295.8	67.2 (-24.0, 158.5)
Hyperbilirubinemia*	1 (1.4)	1 (1.4)	-
Polycythemia^	0	2 (2.8)	-
Hematocrit at 48 (±6) h[‡]	57.4 (4.4)	56.4 (4.8)	1.02 (-1.2, 3.2)

**Requiring phototherapy; [#]at 6 (±1) wk; ^requiring partial exchange transfusion; [‡]Newborns delivered by lower segment caesarean section, n=31 in UCC and 36 in DCC group.*

cut segment of about 30 cm) can transfer nearly 18 mL/kg of blood to the newborn [9,21,22]. The newborn is likely to get more blood if the cord segment is intact, since this allows subsequent refilling of cord from placenta explaining the higher hematocrit at 48 (±6) hours and higher hemoglobin at 6 (±1) weeks seen in the present study.

We observed no difference in the hematocrit in MUC and DCC group in neonates delivered by LSCS. A recent study by Katheria, *et al*. [11] in preterm neonates delivered by cesarean delivery suggested a higher hemoglobin (within the first 24 hours) in MUC group. Infants delivered by cesarean section have a lower

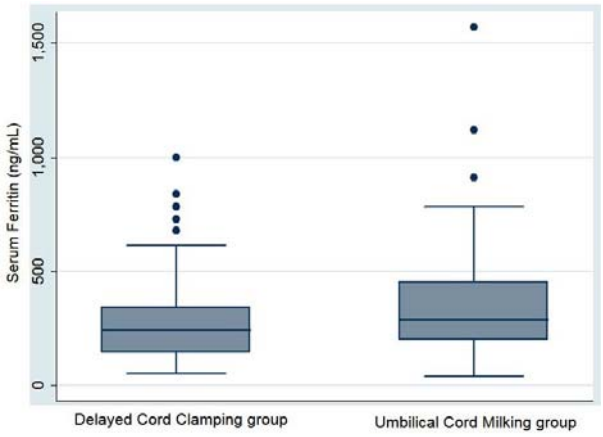


Fig. 2 *Box-and-whisker plot for serum ferritin at 6 (±1) weeks in neonates in the delayed cord clamping and umbilical cords milking groups.*

circulating red cell volume due to the anesthetic and surgical interventions which interfere with active uterine contraction, thus leading to more blood volume remaining in placenta and hence a lower hematocrit [22]. However, we did not evaluate the hematocrit at birth or within 24 hours.

We did not observe any difference in the incidence of hyperbilirubinemia requiring phototherapy or the incidence of polycythemia at 48 (±6) or any difference in serum ferritin at 6 (±1) weeks. These findings have been previously reported [8,9,12,16].

Our study is the first study in late preterm and term neonates where intact umbilical cord milking (milking done with umbilical cord attached to placenta) was compared to delayed cord clamping for evaluation of hematological parameters. This trial ensured appropriate allocation concealment. The outcome assessors and laboratory team were blinded to the intervention arm. We had a follow up rate of 82%. Our study had some limitations too. A longer follow-up till at least 6 to 12 months is desirable to establish whether the initial advantage in hematocrit also translates into gains in infancy and early childhood, which we did not plan.

Umbilical cord milking leads to higher venous hematocrit at 48 (±6) hours when compared with delayed cord clamping in late preterm and term neonates, however long-term effects of milking need to be further evaluated.

Ethics clearance: Institute Ethics Committee, AIIMS; No. IECPG/197/24.02.2016, RT-10, dated March 30, 2016.
Contributors: MKM: protocol development, study implementation, data management and writing the manuscript; AT, MJS: development of the protocol and supervised implementation of the study and contributed to writing of the manuscript and did data analysis; VKP, AKD, RA: protocol development, and provided critical inputs in manuscript writing. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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WHAT IS ALREADY KNOWN?

- Delayed cord clamping leads to improvement in levels of hemoglobin and hematocrit at two months of age.

WHAT THIS STUDY ADDS?

- Umbilical cord milking leads to higher venous hematocrit at 48 (±6) hours when compared with delayed cord clamping in late preterm and term neonates
- Intact cord milking does not result in neonatal hyperbilirubinemia or symptomatic polycythemia as compared to delayed cord clamping.

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