Original Article

Effect of timing of cord clamping on iron stores of infants born to anemic mothers

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Received – 14 June 2015 Initial Review – 25 July 2015 Published Online – 11 September 2015

Abstract

Objective: To study the effect of timing of cord clamping on the iron stores of infants born to anemic (hemoglobin [Hb] 7-10 g/dl) mothers, and if late clamping leads to polycythemia. **Design:** Randomized comparative trial. **Setting:** Tertiary care hospital in a metropolitan city. **Participants:** Term, healthy, and vaginally delivered neonates without any congenital malformations or birth asphyxia, born to booked anemic (Hb 7-10 g/dl) mothers having no medical or pregnancy-related complications were included in the study. **Interventions:** The pregnant mothers were randomized into three groups, and their umbilical cords were clamped at 1, 2, and 3 min. Neonatal hematocrit was estimated by the capillary method at 24 h of life, and infant's (Hb) and ferritin were obtained at 3 months of life. **Main Outcome Measures:** Hematocrit at 24 h of life and serum iron and ferritin levels at 3 months of age. **Results:** The outcome variables significantly associated ($p<0.05$) with hematocrit were cord clamping time, maternal Hb and cord Hb, and ferritin. That associated with infant's Hb were cord clamping time and cord Hb and ferritin. Whereas, only cord clamp time was significantly associated with infant's ferritin. None of the neonates had polycythemia, and Hb and ferritin were found to be the highest in those whose cord was clamped at 3 min. **Conclusion:** In resource-constrained countries, where iron deficiency anemia is a major public health problem, delaying the umbilical cord clamping by up to 3 min will reduce the incidence of infantile anemia. It will serve as an additional cost-effective intervention in anemia control program without any adverse effect of polycythemia.

Key words: *Anemia, Cord clamping, Ferritin, Hematocrit, Hemoglobin*

Anemia is a reduction in hemoglobin concentration (Hb), hematocrit, or number of red blood cells per cubic millimeter. The lower limit of the normal range is set at two standard deviations below the mean for age and sex for the normal population [1]. Anemia long has been recognized as a major public health problem affecting about 10% of the population in developed countries and 25-50% in developing countries. In our country also, it results in significant morbidity and mortality affecting 79.1% of children between 3 and 6 years and 56.2% of married females of 15-49 years age group [2].

Iron deficiency anemia (IDA) is the most common type of anemia. It is the most common nutritional deficiency in children and is worldwide in distribution. It is estimated that 30% of the global population suffers from IDA; most of those affected live in developing countries [3,4]. The amount of iron obtained from the diet during 1st year of life is minimal. So, if the iron stores are low at birth, these children are predisposed to IDA in later infancy. The two possible causes of reduction in the iron content of the newborn are iron deficiency in the mother [5,6] and deprivation of the infant of placental blood by early cord clamping at birth [7].

So, building up the nutritional status of the mother and late clamping of the cord could be two possible interventions in reducing the incidence of IDA in infants. In the case of iron deficient mothers, late cord clamping remains the only possibility. If delayed cord clamping could significantly enhance iron stores of infants born to anemic mothers, it could help in decreasing the prevalence of anemia during infancy and thus, have programmatic implications at the national level.

In spite of, so much work done in this field, there still remains certain lacuna. The optimum timing of cord clamping still remains a controversy, the side effects and benefits associated with late cord clamping if any, and satisfactory Indian data in this regard are still deficient. Therefore, the present study was conducted to evaluate the influence of timing of cord clamping on the iron stores of infants born to moderately anemic mothers and adverse effect of the same, if any.

METHODS

The study was a randomized comparative trial conducted at two labor rooms of a tertiary care unit in New Delhi after approval of Ethical Committee of the institution. Booked

mothers, who had at least three antenatal care [ANC] visits in the hospital, with Hb of 7-10 g/dl without any significant medical or pregnancy-related complications were considered for inclusion in this study. The nature of the study was properly explained, and a printed patient information sheet was also provided. They were included in the study only after obtaining an informed written consent. Sample size was calculated to be 172 on the basis of sample sizes taken in the previous studies with a power of more than 80% and a probability of 5%.

Baseline maternal data (including age, weight, and Hb, number of antenatal visits, obstetric history, and gestation duration) were collected and the timing of cord clamping in a particular case was determined by the randomization. This was determined by the random numbers taken from opaque sealed envelopes and cords were cut accordingly at 1, 2, and 3 min. Healthy term vaginally born neonates without any congenital malformations, birth asphyxia, or indication of neonatal intensive care unit admission were included in the study. After clamping the cord, the baby was left on the maternal chest for initiation of early breast feeding.

At 24 h of life, hematocrit of the neonate was determined on the heel prick sample by capillary method. The proper follow-up of these infants was done at 6 and 10 weeks when they came for routine immunization. At 3 months of age, the infants were called for analysis of Hb (1 ml venous sample collected in EDTA vial and analyzed using cyanide free method of Hb estimation by the machine SYSMEX KX-21) and serum ferritin (2 ml of venous blood collected in iron free vial and analyzed using immunoassay system of estimation using the machine Beckman Coulter Access-2). Baseline infant data were obtained and those not meeting the inclusion criteria at this time were excluded from the study, i.e., those who have received iron supplementation or had blood transfusion in these 3 months. Those infants who had to be admitted for any significant illness in these 3 months were also excluded from the study.

All the statistical analyses were done using SPSS 13.0 except for quantile regression models. These models were applied through Stata 11.0. Frequency distributions were estimated for categorical variables while mean and median along with standard deviation and interquartile range, respectively were estimated for continuous variables to present the distribution of variables. Analysis of variance was used to test the associations between hematocrit and other categorical variables. Association between hematocrit and continuous variables were tested through linear regression models. Variables significantly associated with hematocrit were further adjusted for the association between clamp time and hematocrit. Non-parametric Kruskal–Walis test was used to test the association of infant Hb and ferritin with other categorical variables, $p<0.05$ was considered as statistically significant.

RESULTS

A total number of 233 pregnant females were included in the study initially. Finally, the analysis was carried out on 182 mother-infant pair. About 51 were excluded from the study due to reasons summarized in Fig. 1.

On applying normality test on three outcome variables, neonatal hematocrit was found normally distributed, while infant's Hb and infant's serum ferritin were not normally distributed.

Table 1 represents the association between hematocrit and other variables.

Only clamp time was significantly associated with all the three characteristics, i.e., neonatal Hb, infant's Hb, and ferritin. None other characteristics such as ANC visits, gestational age, gravida, neonatal sex, and exclusive breastfeeding were significantly associated.

From all the predictors, only clamp time, maternal Hb, cord blood Hb, and cord-blood ferritin were found to be significantly associated with hematocrit. Infant Hb was also significantly associated with cord blood Hb ($p=0.032$) and ferritin ($p=0.021$).

DISCUSSION

The present study was a randomized comparative study carried out to see the effect of three different timings (i.e., 1, 2, and 3 min) of cord clamping in infants born to anemic mothers on the iron stores of these infants at 3 months of age and if any of these timings predisposes to polycythemia in these neonates (determined at 24 h of life).

A number of previous studied have been done in different countries of the world comparing the effects of early and late

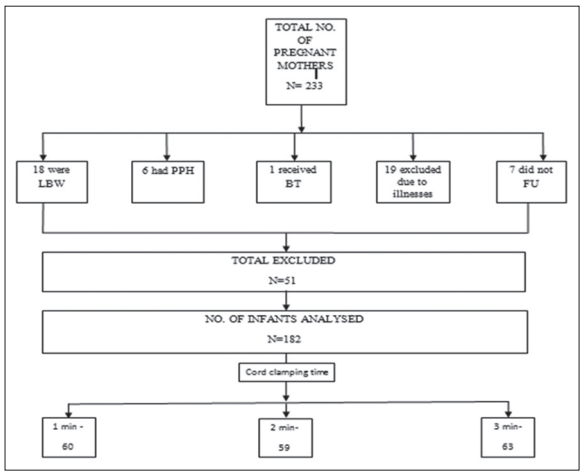

Figure 1: Depicting the causes of exclusion from the study. LBW - Low birth weight, PPH - Post-partum hemorrhage, BT - Blood transfusion, FU - Follow-up

Table 1: Association between different characteristics and neonatal hematocrit, infant Hb and ferritin

Characteristics	Newborn hematocrit (%)		Infant Hb (g/dl)		Infant ferritin	
	Least square mean (95% CI)	p value	Median (IQR)	p value	Median (IQR)	p value
Sex						
Male	56.26 (55.52-57.00)	0.427	9.00 (8.10-10.25)	0.086	71.50 (50.03-95.98)	0.145
Female	55.83 (55.04-56.61)		9.50 (8.20-10.70)		82.95 (50.75-102.43)	
Clamp time						
1	53.82 (52.99-54.65)	<0.001	8.00 (7.80-8.20)	<0.001	47.00 (39.85-50.55)	<0.001
2	56.24 (55.40-57.07)		9.10 (8.90-9.43)		75.65 (67.45-81.50)	
3	58.02 (57.21-58.83)		10.70 (10.30-11.10)		103.80 (97.3-112.80)	
ANC visits						
3	55.67 (54.91-56.44)	0.189	9.20 (8.20-10.55)	0.855	78.40 (50.95-102.65)	0.370
4	55.93 (54.99-56.87)		9.00 (8.15-10.25)		72.40 (50.00-96.20)	
5	57.20 (55.88-58.52)		8.90 (8.20-10.73)		72.65 (52.68-97.05)	
6	57.75 (54.14-61.36)		8.90 (8.33-9.40)		65.80 (45.70-67.45)	
Obstetric history (Gravida)						
1	56.02 (55.05-56.99)	0.913	8.90 (8.20-10.20)	0.932	68.80 (50.35-95.00)	0.855
2	56.31 (55.49-57.12)		9.20 (8.20-10.55)		77.50 (50.75-102.45)	
3	55.61 (54.30-56.93)		9.50 (8.10-10.55)		74.90 (49.20-100.50)	
4	56.60 (53.32-59.88)		9.80 (8.00-11.00)		87.40 (53.05-111.55)	
5	55.00 (52.23-57.77)		10.20 (8.00-10.70)		90.80 (50.70-102.70)	
6	56.00 (48.67-63.33)		8.90 (8.20-10.20)			
Gestation (weeks)						
37	56.59 (55.51-57.66)	0.818	9.60 (8.90-10.55)	0.249	88.30 (67.50-102.90)	0.125
38	55.91 (54.92-56.89)		9.00 (8.20-10.50)		75.60 (47.80-98.60)	
39	56.00 (54.99-57.01)		8.95 (8.18-10.45)		73.80 (48.60-96.28)	
40	55.50 (53.87-57.13)		8.25 (8.03-9.80)		50.70 (47.80-86.60)	
41	55.78 (53.34-58.21)		9.20 (8.05-10.80)		76.90 (56.15-99.05)	
Exclusive breastfeeding						
No	55.71 (54.96-56.47)	0.212	9.10 (8.20-9.90)	0.195	68.55 (50.83-96.50)	0.243
Yes	56.40 (55.64-57.15)		9.20 (8.20-10.70)		82.95 (50.30-99.75)	

CI: Confidence interval, Hb: Hemoglobin, ANC: Antenatal care, IQR: Interquartile range

cord clamping, and invariably majority support the delayed clamping [7-9]. Two Indian studies also supported the benefits of late clamping [10,11]. However, the actual timing of cord clamping is still a dilemma, which the present study has tried to answer to an extent. In most of the previously done studies, cord clamping was done mostly in two groups, i.e. early and late groups. A randomized controlled trial (RCT) was done in Argentina by Jose et al. in 2006 in which they had three cord clamping groups (15 s, 1 and 3 min), like the present study with three groups [12].

The variables significantly associated with hematocrit at 24 h of life were timing of cord clamping, maternal Hb, and cord blood Hb and ferritin. Even after adjusting the other significantly associated variables with hematocrit, the clamp time was found to be significantly associated with hematocrit. Furthermore, the difference of hematocrit between the two groups was statistically significant. The highest value of mean hematocrit (58.08%) was found in infants where cord was clamped at 3 min. Only two neonates had hematocrit of 65%; therefore, none of them had polycythemia. Thus, cord clamping at 3 min had advantage over earlier clamped ones, and it does not increase the risk of polycythemia too.

Variables significantly associated with infant Hb at 3 months of age were timing of cord clamping, and cord blood Hb and ferritin. Even after adjusting the other significantly associated variables with infant Hb, the clamp time was found to be significantly associated with it. The highest value of median Hb of 10.67 g/dl was in those where cord was clamped at 3 min. Even this is favoring the cord clamp time of 3 min, showing its benefits.

Only timing of cord clamping was found to be significantly associated with infant serum ferritin at 3 months of age. The median value of serum ferritin (103.80 μg/L) in group with

Table 2: Maternal and neonatal characteristics and their estimates

Characteristics	Estimate (SE)†	p value	Estimates (SE)††	p value	Estimates (SE)††	p value
Maternal Hb (g/dl)	2.16 (0.30)	<0.001	0.13 (0.14)	0.359	*	*
Cord blood Hb (g/dL)	1.81 (0.11)	<0.001	0.14 (0.07)	0.032	0.87 (2.85)	0.759
Cord blood ferritin (μg/L)	0.14 (0.01)	<0.001	0.012 (0.006)	0.021	0.12 (0.24)	0.609
Maternal age (year)	−0.08 (−0.09)	0.398	*	*	*	*
Maternal weight (kg)	0.07 (0.08)	0.342	0.011 (0.034)	0.751	0.22 (1.09)	0.842
Birth weight (kg)	−0.86 (1.66)	0.606	−0.89 (0.75)	0.238	−26.55 (22.61)	0.242

†Estimates are β coefficients obtained through, ‡linear regression model or ††quantile regression model. *Not sufficient variation in variable to produce estimate. SE: Standard error, Hb: Hemoglobin

cord clamping time of 3 min was significantly more than the value in group with cord clamping time of 2 min (75.65 μg/L) and 1 min (47 μg/L). Thus, clamp timings were significantly associated with all the three outcome variables i.e., hematocrit at 24 h of life and infant Hb and ferritin at 3 months of age making 3 min to be definitely the best time to clamp the cord without any risk of developing polycythemia.

Even randomized controlled trials are now available showing improved neurodevelopmental outcome at later ages in those cord have been clamped. A recent Swedish RCT has shown improved fine motor and social skills at 4 years of age in those where cord was clamped late in comparison to those in whom cord was clamped early [13]. Recent Cochrane review which included 15 RCTs having a large sample size of 3911 women-neonate pairs showed multiple benefits of delayed cord clamping such as improved birth weights, higher early Hb concentrations, and improved iron reserves up to 6 months. However, no significant difference in the rates of post-partum maternal hemorrhage was seen between two groups [14].

Needless to say, the study like any other study had limitations. The study was conducted at a single center. To come out with a consensus, multicentric study needs to be done with large sample size. In this study, both the exclusively breastfed infants and those supplemented with other milk types along with the breast milk were included. The iron absorbed from the breast milk is more than that of other milk types. In this study, only full term healthy neonates were included; however, to generalize the findings of the present study, all types of neonates should be included. The study also did not look into other adverse effects of delayed cord clamping on neonates such as jaundice and moreover no maternal issues were addressed in this study.

CONCLUSION

In resource-constrained countries, where IDA is a major public health problem, delaying the umbilical cord clamping by up to 3 min can be undertaken. It will serve as an additional cost-effective intervention in our anemia prevention or control program. This will also increase the neonatal blood volume without any adverse effect of polycythemia.

ACKNOWLEDGMENT

We thank Mrs. Manpreet Kaur for doing hematological sample analysis.

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Funding: None; Conflict of Interest: None Stated.

How to cite this article: Murtaza K, Aggarwal KC, Kumar A, Jindal M, Batra A. Effect of timing of cord clamping on iron stores of infants born to anemic mothers. Indian J Child Health. 2015;2(3):99-103.