Original Research

Less Residual placental blood volume left when cord pulsation ceases than when early cord clamping at 60 seconds

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Summary

Objectives: Our goal was to test the volume of residual placental blood volume (RPBV) left with delayed cord clamping occurring when pulsation of the umbilical artery ceases as compared to early cord clamping at 60 seconds along with an assessment of its effects on maternal and neonatal outcomes. *Materials and Methods*: From March to June 2015 in Haikou maternal and child health hospital, 403 single-term patients with normal vaginal births and healthy babies were enrolled with randomization into two groups. The experimental group (n = 201) received delayed cord clamping after pulsation of the umbilical artery ceased with the control group (n = 202) having the umbilical cord cut at exactly 60 seconds. RPBV was collected when the cord was cut in both groups. Maternal and neonatal conditions were recorded. *Results*: In the control group, RPBV per birth weight (RPBV (mL/kg) range) [21.0 ± 14.9 (2.9-73.2)] was higher than that in experimental group [4.3 ± 3.5 (0.8 - 19.1)] ($p < 0.01$); peak total serum bilirubin (TSB) level (mg/dL) [(13.6 ± 3.5) (5.0 - 20.7) mg/d1] was higher in the control than that in experimental group [11.9 ± 2.9 (4.5 - 24.5)] ($p < 0.01$); and postpartum blood loss in control group [(187.8 ± 104.6) (80-650.00) mL] was higher than that in experimental group [(160.2 ± 72.9) (70 - 450) mL] ($p = 0.02$). Maternal age, gestational age and Apgar score at one and five minutes were not of statistically different between groups. No neonatal deaths were recorded in either group at one month's follow up. *Conclusions*: Delayed cord clamping until the umbilical artery pulsation ceased is a safe intervention and reduces the residual placental blood volume without any adverse maternal or neonatal effects. The data suggests that more placental transfusion occurs in the newborn with delayed cord clamping.

Key words: Residual placental blood volume (RPBV); Delayed cord clamping (DCC); Early cord clamping (ECC); Placental neonatal transfusion; UCB bank.

Introduction

Delayed umbilical cord clamping, defined as clamping of the umbilical cord until 1 - 3 minutes after birth, appears to be beneficial for term and preterm infants as it increases hemoglobin levels at birth, iron stores in the first several months of life, improved transitional circulation, lower incidence of necrotizing enterocolitis and intraventricular hemorrhage and decreased postpartum hemorrhage of mothers [1, 2]. Report by Pierre Bodin [3] stated that 92 cm^3 of maternal blood was lost after early clamping and that this blood could be saved and passed to the infant with delayed clamping. It has been reported that approximately 30% of fetoplacental blood volume remains in the placenta with immediate cord clamping after birth; it decreases to 20% with a 60 second delay of cord clamping and approximately 13% of fetoplacental blood is left behind if the delay in clamping the umbilical cord extends 3 to 5 minutes [4, 5]. A conflicting problem presents with umbilical cord blood collection for banking. With the discovery of umbilical cord blood (UCB) as a graft source for patients with malignant or genetic diseases in the 1990s [6, 7], UCB banks worldwide were established with large number of UCB units having been donated and stored. [8].

The average amount of blood drawn from the cord per birth is usually higher than 100 mL [9]. This amount is almost half of the total blood volume of the average newborn. The umbilical blood volume required for stem cell therapy is from 75 to 282 mL [5] with a minimum of 120 mL UCB needed for a successful graft implantation [5, 7]. The time of umbilical cord collection for banking occurs almost always immediately after birth. This process of umbilical cord banking may deprive newborns of blood needed at birth for placental transfusion by early cord clamping.

The amount of blood being collected may be influenced by several factors. The time of cord clamping will affect the amount of blood being collected. Alistair found that the residue blood left in the cord in the group with delayed cord clamping for at least 30 seconds (45.14 ± 4.69 mL) was less than that in the early cord clamping group when the cord was cut within 10 seconds of birth (103.14 ± 6.28 mL) ($p < 0.01$) [10].

A critical question is how much blood left in the umbili-

cal cord when the pulsing of the cord ceases? [11, 12]. We believe that it is not an ethical action to draw the umbilical blood while the cord is still pulsating, since the autologous transplantation of stem cells is a natural phenomenon at birth in mammals. A delay in cord clamping can increase stem cell supply to the baby allowing an innate stem cell therapy, that can offer the newborn short term and long-term benefits against age-related diseases [13].

Currently there is no ethical way to measure blood volume of healthy term infants during the first few minutes after birth; thus, measurement of the RPBV can be an easy and safe method used to approximate the amount of blood transfused to the infant [14-16]. Authors have attempted to weigh infants with the attached cord over the first five minutes after birth with the birth weight increasing after delayed cord clamping [17].

The hypothesis of this study is that the residual blood in the umbilical cord when pulsation ceases is much less than clamping the cord while pulsating at exactly 60 seconds. Early clamping and collecting the blood from the cord before pulsation ceases is draining excessive blood from the newborn that should be transferred to their own body.

Materials and Methods

This was a prospective, unblinded, two-group randomized controlled trial conducted in Haikou maternal and neonate hospital between March to June 2015. Women and their babies meeting the inclusion criteria were enrolled. The experimental group (n = 201) received delayed cord clamping that at the arrest of the pulsation of cord and the control group (n = 202) including neonates with cord clamping at 60 seconds. Residual blood remaining in the cord and placenta was collected and measured. Apgar scores, birth weight, daily bilirubin level, maternal age, weeks gestation, hemoglobin levels and postpartum hemorrhage were recorded.

Inclusion criteria included women with a healthy uncomplicated pregnancy, no diagnosed medical diseases, singleton fetus in longitudinal position, cephalic presentation, spontaneous onset of labor, gestational weeks 37 + 0 to 41 + 6, vaginal birth, Apgar score > 7, birth weight > 2,500 grams, and absence of postpartum hemorrhage. The placenta delivered spontaneously and was intact.

Exclusion criteria included pregnancy related complications such as hypertension, diabetes, assisted delivery, cesarean section, postpartum hemorrhage, preterm, twins, breach delivery, Apgar score less than 7, and congenital malformations.

The midwife in charge confirmed the woman's eligibility for participation in the trial according to the criteria at the time of admission to the delivery ward.

A report Alistair [10] demonstrated that the residual blood volume left in the cord in the group with delayed cord clamping for at least 30 seconds was (45.14 ± 4.69)

mL, and in early cord clamping group with cord cutting occurring within 10 seconds of delivery was (103.14 ± 6.2) mL with the mean difference between groups being 58 mL. According to this results, to achieve a strength of 80% at a significance level of 0.05 (two-sided) fewer than 10 cases would be required [20]. Our goal was to recruit as many participants as possible during the data collection period.

Figure 1 is the CONSORT flowchart. Out of a total of 1,808 women assessed for the study, 460 were ineligible and excluded due to high-risk pregnancy, assisted delivery, epidural anesthesia, spontaneous rupture of membranes and cesarean sections. Of the 1,348 participants eligible for this study, 440 signed an informed consent form and were recruited to this study. In total, 403 women completed the protocol with 201 in the experimental group and 202 in the control group with 37 withdrawing from the study. Randomization occurred by assigned numbers being place into opaque and sealed envelopes and placed in the labor wards of the hospitals. A data collection sheet was enclosed in every envelope to collect the women's demographic and other data. When a woman was admitted into the labor room, the midwife asked whether the woman was still willing to participate. Upon receiving patient consent, the midwife drew an envelope in strict numerical succession prior to delivery.

The principal investigator (Ms. ZHY) and trial coordinators (Ms. WY and GRF) gave presentations to the obstetric nursing staff at the hospital to ensure that everyone understood the protocol and a trained research nurse who was not be involved in assisting delivery was response for collection of the data.

The women in the experimental group received delayed cord clamping protocol with the cord not being clamped and cut until pulsation ceased. The participants in the control group received early cord clamping with the cord being clamped at 60 seconds after birth.

The primary measured outcome was the residual blood volume between two groups, the peak bilirubin level of each infant, and the likelihood of postpartum hemorrhage. Residual blood left in the cord was collected after clamping of the cord in each group (stop of pulsation in experimental group and at 60 seconds in the control group). The cord was clamped with two instruments with blood in the cord and placenta being drained into a tube with liter scale mark while the placenta was still attached to the uterus. When the placenta was delivered, the health worker held in place the placenta at a height of 30 cm until blood dripping stopped. The collection method reported by Redmond placed the placenta at an equipment height of 46 cm and drained the blood through gravity [14]. The present authors did not accept this method because majority of the placentas did not deliver at the time of cord clamping.

Bilirubin level of the newborns were measured with transcutaneous bilirubinometer daily until seven days after birth and the peak bilirubin levels were analyzed between the

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│   Total number of participant   │
│   during study period (1808)    │
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        │  460 ineligible for study    │
        │  1348 eligible for study     │
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    │  440 signed inform content      │
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│ In experimental      │  │ In control group     │
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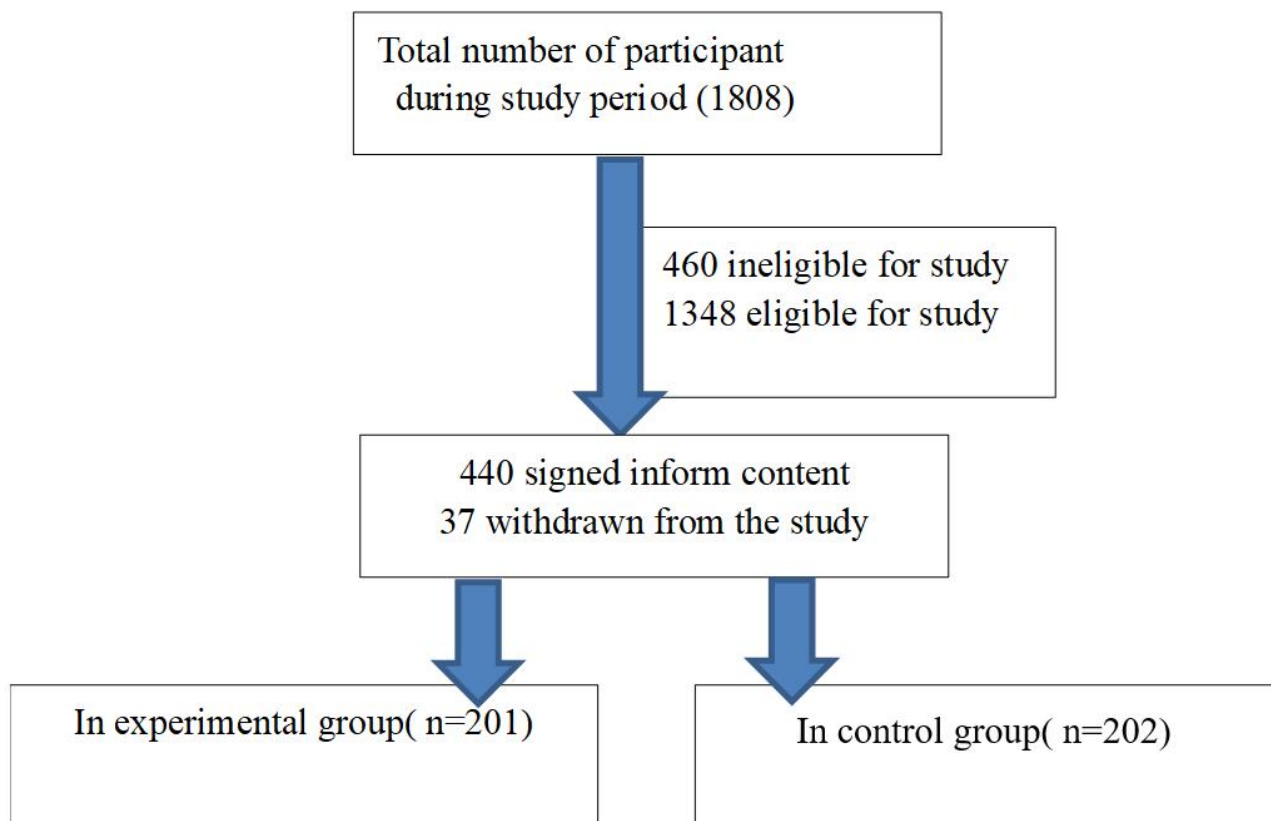
Figure 1. — Consort data.

groups. Postpartum blood loss was weighed for the first 24 hours after birth.

Ethical approval was obtained from the Health Research Ethics Boards of the participating hospitals. The midwives working in the delivery room provided written and oral information to mothers and explained the purpose and nature of the research, ensured the confidentiality of data collected, anonymity and the maternal right to refuse participation. Written consent was signed by every woman willing to participate in the study.

They were also informed of their freedom to withdraw from this study without any interference on their obstetric care.

The data collected were analyzed by SPSS Statistics for version 2.1. A per-protocol analysis was performed on outcome data that was available for women who completed the study protocol. Descriptive statistics were used to report demographic information of the participants. Data are given as means and standard deviation (SD). Independent sample t-tests and Mann-Whitney U tests were used to compare results of continuous data of gestational week, age, neonatal birth weight, residual blood in the placenta, amount of postpartum bleeding and daily bilirubin level of each baby between the two groups. Chi-square tests were used to examine the differences of variables between the two groups for categorical data. The level of statistical significance was set at $p < 0.05$ and all inferential tests were two-tailed.

Data were collected between May 2015 to June 2015 in Haikou maternal and neonatal hospital in China. In total, 403 women completed the protocol with, 202 mothers completing the protocol with the delayed cord clamping in the experimental group and 201 mothers in the control group.

The maternal demographic characteristics are shown in Table 1. There was no difference in maternal age and parity between groups and postpartum blood loss was less in experimental group [(160.2 ± 72.9) (70 - 450) mL] than that in control group [(187.8 ± 104.6) (80 - 650.00 mL] ($p = 0.02$) (Table 1).

Neonatal conditions between groups is shown in Table 2. No difference was found in gestational age, gender and Apgar scores at 1 and 5 minutes. The experimental group had lower birth weight and lower peak bilirubin levels than in the control group. Residual blood left in the placenta and cord less in the experimental group (14.1 ± 12.4 mL) than that in control group (69.0 ± 50.0 mL) with the same results being seen in the residual blood per birth weight being less in the experimental group [(4.3 ± 3.5) (0.8 - 19.1) mL] than that in the control group [(21.0 ± 14.9) (2.9 - 73.2) mL] (Table 2).

Table 1. — *Maternal Demographics and Clinical Variables at Birth*

Maternal Characteristics	DCC (n = 201)	ICC (n = 202)	*P* value
Age (years)	31.5 ± 5.6 (17 - 54)	32.2 5.5 (20 - 49)	0.18
Primipara	72 (35)	75 (37)	0.78
Postpartum bleeding (mL) range	160.2 ± 72.9 (70 - 450)	187.7 ± 104.6 (80 - 650.00)	0.02

N (%) or mean ± SD (range)

Table 2. — *Infant Demographics and Clinical Variables at Birth*

Infant Characteristics	DCC (n = 201)	ICC (n = 202)	*P* value
Gestational Age, weeks	38.4 ± 1 (37 - 42)	38.3 ± 1 (37 - 43)	0.36
Cord Clamping Time (sec) Range	113.9 ± 85.9 (18 - 540)	60 (60)	—
Apgar Scores 1 minute,	9.9 (9 - 10)	9.9 (8 - 10)	0.1
median (range) 5 minutes	10 (10 - 10)	10 (10 - 10)	—
Male/female	106/95	110/92	0.72
Birth Weight (g) Range	3211.4 ± 423.2 (2200 - 4900)	3297.2 ± 386.8 (2300 - 4600)	0.035
RPBV (mL) Range	14.1 ± 12.4 (2 - 100)	69.0 ± 50.1 (10 - 205)	0
RPBV (mL/kg) Range	4.3 ± 3.5 (0.8 - 19.1)	21.0 ± 14.9 (2.9 - 73.2)	0
Peak total serum bilirubin (TSB) level, (mg/dL)*	11.9 ± 2.9 (4.5 - 24.5)	13.6 ± 3.5 (5.0 - 20.7)	0

N (%) or mean ± SD (range)

Discussion

Clamping the cord after pulsation ceases reduces the blood left in the cord and placenta resulting in more blood being transferred to the newborn. The transfusion process from placenta to the newborn immediately after birth is an essential step to convert a fetus to newborns; 80 to 100 mL blood is expected to be transferred during this period. It is simpler and easier to observe how much blood would be left in the cord and placenta than to quantify how much blood is transferred which was the aim of this study.

The optimal time of cord clamping varies internationally. The World Health Organization recommends a one- to three-minute delay [1], while the American College of Obstetricians and Gynecologists recommends a delay in umbilical cord clamping in term and preterm infants for no less than 30 - 60 seconds after birth [12]. In this study, delayed cord clamping in the experimental group was conducted until the pulsation ceased, which was an individual time interval for each baby transfer process [(113.9 ± 85.9) (18 - 540) seconds]. The control group included clamping the cord at 60 seconds after birth to avoid the potential harmful effect of early cord clamping of less than 60 seconds. The amount of residual blood in the experimental group was (14.1 ± 12.4) mL and less than that in control group (69.0 ± 50.0) mL. This result is consistent with Alistair's study where the residual blood in 30 second was (45.14 ± 4.69) mL, and in the 10-second group it was (103.14 ± 6.28) mL. Since the baby's weight may influence the volume of blood, it is more logical to make a comparison via birth weight. The result RPBV per birth weight (RPBV (mL/kg) range) in ex-

perimental group [4.3 ± 3.5 (0.8 - 19.1) mL] was less than that in the control group [21.0 ± 14.9 (2.9 - 73.2) mL] (*p* < 0.01).

A recent study by Katheria et al. [4] reported the RPBV (mL/kg) range while waiting 5 minutes by placing the baby on mother's abdomen was [20.0 ± 8.5 (0 - 36.8) (mL/kg)] and in the early clamping group (≤ 20 seconds) it was [30.8 ± 9.6 (14.1 - 65.2) (mL/kg)]. These results may indicate that the time of complete transfusion is not 5 minutes but until cord pulsation ceases. The longest time of pulsation was 540 seconds.

The present research offers new information to the process of placental transfusion with more time being required to complete transfusion process.

Several studies have indicated that blood retained in the placenta is in relation to the onset of respiration for the newborn; when respiration was established before cord clamping, less blood was retained within the placenta (and by inference, greater placental transfusion occurred) [14-16].

In the present study, both groups had the cord clamped after establishing regular breathing; hence the inference of breathing on the effect of cord blood transfusion can be minimized.

Furthermore, the position of the infant following delivery could also affect placental transfusion to the baby. A Previous study in term babies positioned babies either lower than the introitus [17] or at the same level [18]. In the present study babies in both groups were placed at the same level on the delivery bed after birth negating the effect of gravity.

The cord blood banking process may draw too much

blood from the newborn through earlier clamping of the cord. It was reported that the average volume of cord blood collected for banking ranges from approximately 75 to 125 mL [19, 20], and the authors suggested earlier clamping for more blood. A recent study in Sahlgrenska University Hospital/Östra showed that the amount of blood collected with early cord clamping ($<$ 15 seconds after birth of the infant) was 119 mL (median 118 mL, SD 28 mL), but the amount of blood collected after for clamping 60 seconds after birth was 111 mL (median 109 mL, SD 32 mL).which was not statistically significant [21]. This result is in contrast to the observation of the present authors that the time to collect blood in the delayed group requires further evaluation.

No adverse effects were observed by clamping the cord until pulsation ceased except for lower blood loss of the mothers and lower bilirubin level in newborns. Another important concern of delayed cord clamping was the higher bilirubin level that was assumed to be a consequence of "to much blood" being transferred to the newborns; the data do not support this argument.

What can be determined in this review is that the delayed cord clamping group had a lower rate of need for manual removal of the placenta (RR 0.45 (0.22, 0.94) with, and no adverse effect being s observed in the rate of hyperbilirubinemia or jaundice (5 trials, 2,210 cases, effect size (95% CI) RR 1.16 (0.92, 1.45), neonates requiring phototherapy (5 trials, 1,974 cases, RR 1.28 (0.48, 3.42)) or the presence of polycythemia (6 trials, 936 cases, RR 1.22 (0.79, 1.87)). The rate of maternal postpartum blood loss was not significantly different (4 trials, 1,878 cases, RR 0.82 (0.65, 1.04)) [24]. In the present study, the authors found lower blood loss and levels of bilirubin in newborns in the experimental group that supported the evidence that delayed cord clamping until pulsation ceased had no adverse effect on mothers and babies; however, the beneficial effects of the reduced postpartum blood loss and lower level of bilirubin require further study.

Conclusion

Less blood left in the umbilical cord and placenta when clamping the cord after pulsation ceases indicates that more blood is transferred to the newborn. No adverse effects were observed in mothers or newborns except for a lower postpartum blood loss and lower bilirubin level.

Scientific comments – This manuscript requires much scientific editing. The authors should simplify their hypothesis with a shortened, manuscript and only discuss their findings as related to delayed cord clamping. All discussion of cord blood banking should be removed as it has nothing to do with the study and presents their bias against this procedure of which I agree it has little use. The data collected is useful to prove the benefits of delayed cord clamping and this is solely what the authors should focus upon.

Conflict of interest

There is no conflict of interest to declare.

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