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Effect of Cord Clamping Timing on Hemoglobin, Hematocrit, MCV and MCHC among Neonates in a Tertiary Institution.

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Abstract:
Background: *The timing of cord clamping significantly impacts the total blood volume of the newborn at delivery and varies from hospital to hospital. Hence, this study attempts to elucidate how cord clamping practices affect the red cell indices of term newborns, by determining the effect of timing of cord clamping on red cell indices of neonates born between 37-42 completed weeks of gestational age.*
Materials and Methods: *An analytical cohort study on 296 term newborns at the University of Abuja Teaching Hospital from 5th May to 10th November 2015. 150 had immediate cord clamping and 146 were delayed. Hemoglobin, hematocrit, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were measured from cord blood and venous blood.*
Results*:While cord blood red cell indices were similar for both groups, delayed clamping group had significantly higher hemoglobin and hematocrit values (17.0(2.0) and 55.1(6.0,) respectively) in comparison to immediate cord clamping group (15.0(1.9) and 48.7(5.9), respectively) at 24 hours of life. No significant difference were seen in MCV and MCHC. Further analysis showed significant and proportional increment in the hemoglobin and hematocrit values with increasing delay in timing of cord clamping ($p<0.05$).*
Conclusion*Delayed cord clamping conferred higher hemoglobin and hematocrit values with no significant harmful effect. No difference noted with MCV and MCHC.*
Key Word*: Hemoglobin, Hematocrit, Cord clamping, Placental transfusion.*

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I. Introduction

The umbilical cord is traditionally severed at birth.[1] This intervention denies the newborn of significant blood volume if placental transfusion(extrauterine transfer of blood from placenta to baby) is not permitted.[2] The timing of cord clamping affects the total blood volume of the neonate.[3] Under normal conditions, the blood volume of the newborn is about 70ml/kg, and the placenta contains about 45ml/kg of fetal blood.[4] With placental transfusion, approximately 20 -35ml/kg of blood can be transfused, increasing the newborn's blood volume by up to 50%,[4] with concomitant enhanced oxygen carrying capacity.[5] Cord clamping is classified as immediate (early) or delayed (late) depending on how much time elapses between delivery and application of the first clamp on the cord.[6] Clamping done within first 60 seconds of delivery is termed immediate while beyond 1 minute is delayed.[6,7]

Several studies have been carried out to elucidate the effect of delayed cord clamping on both term and preterm babies, including but not limited to fewer incidences of anemia, intraventricular hemorrhage, early onset sepsis in neonatal life,[8] and iron deficiency anemia in infancy.[9] And current neonatal resuscitation protocols recommend delayed cord clamping.[10] Despite all this, policies on cord clamping practices still vary in different regions.

II. Material And Methods

It was an analytical cohort study carried out in the delivery suites of the Department of Obstetrics and Gynecology, the post-natal ward and the Special Care Baby Unit of the University of Abuja Teaching Hospital within a six month period (May 2015 to November 2015).
Study Design: It was an analytical cohort study
Study Location: The delivery suites of the Department of Obstetrics and Gynecology, the post-natal ward and the Special Care Baby Unit of the University of Abuja Teaching Hospital, Gwagwalada, Abuja.
Study Duration: May 2015 to November 2015.
Sample size: 300 patients.
Sample size calculation: The sample size was calculated using the formula below for comparison of numerical data and based on certain assumptions which were

1. The desired sample size, n
2. A standard deviation about the mean, σ.
3. The difference between the two means, δ
4. A confidence level of 95%, thus Zα of 1.96
5. And Zβ as a constant of 0.8

After correction for sampling population less than 10,000 and attrition factor, a sample size of 126 was achieved. Thus, 150 babies were recruited into each arm (early and late cord clamping).

The **Subjects & selection method**: all babies delivered within the study time frame was considered for the study. However, only those who satisfied our inclusion criteria and had no reason to be excluded was coopted into the study.

Inclusion criteria:
1. All newborns delivered at ≥37 completed weeks of gestation in UATH, Gwagwalada.
2. Uncomplicated deliveries
3. Newborns for whom signed consent was given by their parents.

Exclusion criteria:
1. All newborns delivered via caesarean section.
2. All newborns delivered after 42 completed weeks' gestation.
3. Congenitally malformed babies.
4. Newborns who required immediate resuscitation.
5. Presence of risk factors for anaemia, polycythaemia and hyperbilirubinaemia e.g. antepartum haemorrhage, infants of diabetic mothers, ABO or Rh incompatibility etc.

Procedure methodology

It was an analytical cohort study carried out in the delivery suites of the Department of Obstetrics and Gynecology, the post-natal ward and the Special Care Baby Unit of the University of Abuja Teaching Hospital within a six month period (May 2015 to November 2015). A total of 300 term newborn were recruited into the study after satisfying the inclusion criteria (uncomplicated vaginal delivery at ≥ 37 completed weeks gestational age). Exclusion criteria included delivery beyond 42 completed weeks' gestation, congenitally malformed babies, need for immediate resuscitation and presence of risk factors for anemia, polycythemia and hyperbilirubinemia e.g. antepartum hemorrhage, infants of diabetic mothers, ABO or Rh incompatibility. Four samples were not analyzed (clotted) hence only 296 completed the study and were analyzed. Ethical approval was given by the hospital ethics committee.

All mothers who signed consent to participate were systematically randomized into either of two group by way of picking a closed slip of paper from an unmarked jar. Each slip had been labelled with either the letter A (for immediate clamping) or B (for delayed clamping).The mother reserved the right to know which group she fell into. The timing for ICC group was within 30 seconds of delivery while the timing for the DCC group was within 60-90 seconds post-delivery.

Two to three millilitres of blood was taken from the cord at birth, and one to two millilitres from the peripheral veins of the babies within the first 24 hours of life in accordance with standard aseptic procedure. The cord and venous blood samples obtained were placed in separate EDTA bottles prior to analysis. Red cell indices (hemoglobin, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin concentration) were determined from cord blood and the baby's venous blood sample.

Other essential parameters were recorded, including but not limited to Apgar score, birth weight, length, head circumference and mother's biodata. Social status was determined using Olusanya classification. The questionnaire was administered by the investigator once consent to participate in the study was obtained from the respondents.

Statistical analysis

Data was analyzed using SPSS version 20 (SPSS Inc., Chicago, IL). The means and standard deviation (SD) were computed for the quantitative variables (haemoglobin, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin concentration). The difference between the two means (values for immediate cord clamping versus delayed cord clamping) for each red cell variable were compared using the Student t test, while difference between proportions (e.g. gender, occupation, social status) were compared using chi-square test. Analysis of variance (ANOVA) was used to compare the means among greater than two groups, with the aim to determine any significant effect on the red cell indices with increasing duration of placental transfusion. P value <0.05 was considered as significant. Confidence interval of 95% was assumed.

III. Result

A total of 634 vaginal live births occurred during the period of the study, and 300 were enrolled. 296 were analyzed as 4 samples were invalid. Table one depicts maternal characteristics while table 2 show newborn characteristics. There were no significant difference among the two groups. Cord blood analysis showed no significant difference in all indices between both groups (table 3) however at 24 hours of life, there was significant increase in the hemoglobin and hematocrit values for those who had delayed cord clamping as depicted in table 4 with p value < 0.001. There was no significant difference found for mean corpuscular volume and mean corpuscular haemoglobin concentration (table 4).

Table I: Maternal characteristics.

Characteristics N = 296	**Cord clamping method ICC n =150**	**DCC n = 146**	**p-value**	
	Mean(SD)	Mean(SD)		CI
Age	30.2(4.5)	29.4(4.7)	0.186	-0.282-1.821
Weight	77.3(12.2)	75.9(12.4)	0.522	-1.403-4.221
Height	161.9(5.8)	161.2(5.8)	0.274	-0.609-2.011
	n (%)	n (%)		
Parity				OR (CI)
Primiparity	37(12.5)	42(14.1)	0.434	0.811(0.484-1.358)
Multiparity	97(32.8)	90(30.4)	0.631	0.139(0.710-1.827)
Grandmultiparity	16(5.4)	14(4.8)	0.848	1.126(0.528-2.399)
Tribe				
Yoruba	18(6)	13(4.4)		
Hausa	8(2.7)	13(4.4)		
Igbo	44(14.9)	47(15.9)		
Gbagyi	4(1.4)	5(1.7)		
Others	76(25.6)	68(30)		
Social class				
Upper	59(19.9)	53(18)	0.632	1.138(0.711-1.821)
Middle	62(20.9)	67(22.6)	0.482	0.831(0.524-1.316)
Lower	29(9.8)	26(8.8)	0.767	1.106(0.615-1.989)

Key: N- Total number of subjects, n- number of subjects in each group, SD- standard deviation, OR – odds ratio, CI – confidence interval.

Table II: Newborn characteristics

Characteristics N = 296	**Cord clamping method ICC n = 150**	**DCC n = 146**		
	Mean (SD)	Mean (SD)	p	CI
Clamping time (secs)	7.5 (4.5)	69.4 (10)	< 0.001*	-63.704- -60.170
Gestational age (weeks)	39.1(1.2)	39.2 (1.2)	0.670	-0.336-0.220
Anthropometry				
Weight (gm)	3185 (375.4)	3175 (379.3)	0.832	77.010-95.640
Length (cm)	50.5 (1.8)	50.6 (1.8)	0.555	-0.534-0.287
Head circumference (cm)	34.4 (1.0)	34.4 (1.0)	0.849	-0.212-0.257
	n (%)	n (%)		OR (CI)
Gender				
Male	88 (29.8)	84 (28.4)	0.906	1.048(0.660-1.663)
Female	62 (20.9)	62 (20.9	0.906	1.048(0.660-1.663)

Key: N- Total number of subjects, n- number of subjects in each group, SD- standard deviation, * p < 0.05, OR – odds ratio, CI – confidence interval.

Table III: Cord blood red cell indices versus cord clamping time

Red cell indices	**Cord clamping met ICC Mean(SD) n= 150**	**DCC Mean(SD) n= 146**	**p-value**	CI
Hct(%)	42.8(5.2)	42.4(5.4)	0.662	-0.83985- 1.57756

Hb (g/dL)	13.0(1.6)	12.8(1.9)	0.440	-0.17255 -0.63591
MCV(fl)	109.4(8.2)	109.6(7.5)	0.852	-1.94010 -1.63856
MCHC(g/dl)	30.2(1.5)	30.2(1.7)	0.806	-0.36763-0.37521

Key: Hct – hematocrit, Hb- hemoglobin, MCV- mean corpuscular volume, MCHC- mean corpuscular hemoglobin, SD- standard deviation, CI – confidence interval.

Table IV: Effect of timing on red cell indices at 24 hours of life

Red cell indices N=296	Cord clamping ICC n= 150	DCC n=146		
	mean(SD)	mean (SD)	p	CI
Hct (%)	48.7(5.9)	55.1(6.0)	<0.001*	-7.751- -5.027
Hb (g/dL)	15.0(1.9)	17.0(2.0)	<0.001*	-2.429 - -2.429
MCV (fL)	106.6(11.6)	106.1(11.5)	0.681	-2.182 - 3.113
MCHC (g/dL)	31.0(1.7)	30.9(1.6)	0.709	-0.280 - 0.461

Key: Hct – hematocrit, Hb- hemoglobin, MCV- mean corpuscular volume, MCHC- mean corpuscular hemoglobin, SD- standard deviation, * - p < 0.05, CI – confidence interval.

Following the evidence that delaying cord clamping significantly improves the hemoglobin, hematocrit and oxygen saturation, further analysis using Analysis of Variance was used to determine if longer duration of placental transfusion gave better red cell indices and oxygen saturation. This was achieved by subdividing the delayed cord clamping group into three groups (60-69secs. 70-79 secs and 80-90 seconds) and the comparison is shown in Tables V. Those who had longer delay prior to cord clamping had higher hemoglobin and hematocrit values as depicted in the table. As was previously demonstrated, there was no significance with respect to mean corpuscular volume and mean corpuscular hematocrit concentrations with increasing delay.

Table V: Relationship of increased timing on red cell indices

Red cell indices	**Timing(seconds)** **60-69 Mean(SD) n = 81**	**70-79 Mean(SD) n = 30**	**80-90 Mean(SD) n = 31**	**F**	**p-value**
Hb	16.6(1.9)	17.7(1.6)	17.2(2.3)	3.965	0.021*
Hct	54.0(5.8)	56.8(5.6)	56.4(6.6)	3.423	0.035*
MCV	105.2(13.8)	107.9(8.5)	106.7(5.7)	0.642	0.528
MCHC	30.8(1.5)	31.5(1.5)	30.7(1.7)	0.682	0.122

Key: Hct – hematocrit, Hb- hemoglobin, MCV- mean corpuscular volume, MCHC- mean corpuscular hemoglobin, SD- standard deviation, * - p < 0.05.

IV. Discussion

The study set out to assess how red cell indices are affected by the time it takes to clamp the cord. We were able to show a significant difference in the values for hemoglobin and hematocrit with higher values in those who had delayed cord clamping. This is in line with several studies done previously in term neonates,[11–15] We were able to further show that the longer the delay before clamping, the better the red cell indices, There was no significant difference in relation to mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). None of the aforementioned studies considered MCV and MCHC previously.

It is important to note that while the mean corpuscular volume and mean corpuscular hemoglobin concentration values were similar irrespective of the cord clamping timing, there was a difference between cord blood values and postnatal venous blood values of the babies.

The mean corpuscular volume is an average of the various red cells within the body, and since the newborn cells are large (macrocytic) and lose volume within the first few hours,[16] the mean corpuscular volume decreases postnatally, a phenomenon which was clearly depicted in this study. Consequently, as this volume loss alters the water content of the cell with a resultant reduction in cell volume, and haemoglobin remains constant, the mean corpuscular hemoglobin concentration increases slightly. This was also clearly depicted in this study.

The sample size was considered to be large enough for a comparative study as determined with the formula for comparison of numerical data with standard deviation about the means taken from the study by Emhamed et al in Libya, which was the closest similar study done.[11]

The choice of timing intervals were also considered to be appropriate based on the analysis by a Cochrane review done in 2013 and the current neonatal resuscitation guidelines for delaying cord clamping beyond a minute. [10,17]. While it was not the focus of this study, it was however noted that infants with hematocrit

>65 did not require any intervention. This study however did not follow up the patients beyond the first two days of life. Further studies to determine risk for jaundice and iron status would be needed.

V. Conclusion

Despite recommendations for delayed cord clamping and its elucidated positive effects, early cord clamping still remains the norm in our setting, This study has been able to outline the fact that delaying cord clamping has advantages to the new born, mainly by increasing the hemoglobin and hematocrit of the newborn, and this has the attendant advantages of reducing risk of anemia in the neonatal period, and the increased red cell mass serves as an iron reservoir for the neonate. This safe, cost effective and easy to apply intervention should be incorporated into routine newborn care especially in the developing world as this has the capacity to positively impact our neonatal morbidity and mortality statistics.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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