Umbilical cord blood hemogram – what is the effect of maternal anemia on the fetus?

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Abstract

Purpose: Umbilical cord blood (UCB), a source of hematopoietic stem cells, represents neonatal blood. We had already established a biological reference interval for the UCB hemogram. This study is undertaken to understand the effect of maternal anemia on the fetus. **Materials and Methods:** 100 full term infants born naturally with normal birth weight and APGAR score were enrolled. Maternal haemoglobin cut offs of 12 g/dL and 10.9 g/dL were used to group anemia. UCB was collected after clamping the cord in a K_2 -EDTA evacuated tube and processed in Beckman Coulter LH 780 hematology analyzer. Delta check and manual count of red blood cell (RBC) precursors were done by peripheral smear and supravital methods. Data was analysed using SPSS IBM statistics software version 19. The RBC parameters and RBC precursors were compared between groups and also with the biological reference interval. Correlation of maternal and fetal hemoglobin (Hb) was done. **Results:** No significant difference was found in the RBC parameters and nucleated red blood cell (NRBC) count in the UCB of neonates born to anemic and non-anemic mothers. Reticulocyte parameters showed significant increase in the UCB of neonates born to anemic mothers. When compared to the biological reference interval, red cell hemoglobin was lower and reticulocyte parameters were higher in the UCB of neonates born to anemic mothers. A significant positive Pearson correlation was found between cord blood Hb and maternal Hb. **Conclusion:** Though maternal anemia does not cause fetal anemia, depending on the severity, it causes chronic hypoxia so that the fetal bone marrow reacts to the effect of erythropoietin by increased erythropoiesis and release of RBC precursors into the blood.
Keywords: Fetal RBC, Hemogram, maternal anemia, UCB.

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INTRODUCTION

Umbilical cord blood (UCB) can be used as an alternative to determine the health status of new born. In the recent years, there have been many studies in the cord blood parameters and their applicability to assess the health status of the new born[1] The first published report on the UCBhaemogram was done in 1924 by Lippmann. New born UCB blood parameters vary due to gender, race, socio-economic status of the parents, type of the sample used, methods used for analysis and time of blood sample collection etc[2,3]. Therefore, biological reference interval for complete haemogram on cord blood was established in our centre. Several works had been done in the developed countries on the cord blood stem cells and their applications for curing certain disorders and storage of cord blood collected at time of birth[3]. Analyzing the complete haemogram and comparing with the biological reference interval is essential before cryopreserving the cord blood units. There have been several published data that the cord blood parameters of the neonate vary according to mother's condition.[4] Anemia is the commonest hematologic condition prevalent in pregnant women in developing countries. Researchers have mentioned increased precursors of RBC in term neonates as an indicator of chronic intrauterine hypoxia and the

intensity depends on the duration of asphyxia[5]. The impact of maternal anemia in the newborn is a matter of concern, especially in developing countries like India. It is essential to study the effect of maternal anemia on the UCB parameters.

AIM AND OBJECTIVE

Comparison of RBC parameters and RBC precursors in the umbilical cord blood of neonates born to anemic and non anemic mothers.

MATERIALS AND METHODS

This is a cross sectional comparative study done on 100 full term (37- 42 weeks) babies with normal birth weight [2.5 to 4.0 kgs] and normal apgar score without any congenital anomaly born by natural delivery to booked pregnant women between 21-40 years, who are not smokers and without any co morbid conditions. The cut off hemoglobin chosen is 12 gm% as all our pregnant women were booked cases and were on supplementary iron therapy. However with the Hb value of 10.9gms% also, the anemic and non anemic mothers were grouped.[6] Institutional ethics committee approved this study. The umbilical cord was clamped immediately after the delivery, as a routine by obstetrician. 3ml of UCB was be taken from the cord which attached to the placenta by milking and collected into pre labeled evacuated tube containing ethylene diamine tetra acetic acid (K_2EDTA). Tube was inverted 3-4 times to allow mixing of the blood with anticoagulant. The sample was then transported immediately to the clinical pathology laboratory for processing. The sample was processed in automated hematology analyzer Beckman Coulter LH 780 within a maximum of 2 hours. Peripheral smear was done and NRBCs were counted /No of 100 WBC and also delta check was done. Reticulocyte count was done manually by doing supravital staining using 1% brilliant cresyl blue. Reticulocyte percentage, absolute reticulocyte count, reticulocyte index and reticulocyte production index were calculated. The mean and standard deviation were calculated using SPSS statistics IBM software version 19. Levene's test for equality of variations was done. Attempts were made to determine any significant difference between the groups using the student's t-test. Pearson correlation was used for comparing maternal and UCB Hb value. A probability value (p-value) of less than 0.05 was considered statistically significant.

RESULTS

The mean age of mothers were 25.6 ± 3.6 years ranging from 19 to 35 years. The neonates were full term, with 58 males and 42 females and mean birth weight 2.98 ± 0.43. The mean birth weight of neonates were 3.03 ± 0.37, 2.94 ± 0.46, and 2.68 ± 0.48 for non anemic mothers, anemic mothers with <12gms% and<10.9gms% respectively. The mean $\pm SD$ of each parameter was tabulated and the p-value calculated (Table-1). No significant difference in RBC parameters of UCB of neonates was found between the two groups (p-value >0.05). Significant differences were found in reticulocyte count, absolute reticulocyte count corrected reticulocyte count, and reticulocyte production index (p value= < 0.05) in the UCB of neonates born to anemic and non anemic mothers. However no significant difference was found in the NRBCcount between the two groups both by automation and manual methods (p value > 0.05). A significant decrease in the values of mean corpuscular hemoglobin(MCH) and mean corpuscular hemoglobin concentration(MCHC) in the UCB of neonates born to anemic mothers (Hb <12 gms/dl) with respect to the biological reference interval. No significant increase was found in the NRBC of the UCB of neonates born to anemic mothers when compared with the biological reference interval[7]. Though the NRBC count in the UCB of neonates born to mothers with lower Hb (≤ 10.9 gms %) is more than that of the NRBC count in UCB of neonates born to mothers with Hb of ≤ 12 gms %, difference is statistically not significant (Table-2). The maternal Hb was grouped in to 6 categories with 1gms difference ranging from 8gms to 13gms and the respective Hb value of the neonates were compared in Figure-1. There was a significant positive Pearson correlation between the maternal Hb and cord blood Hb values.

Table 1: Comparison of RBC parameters and precursors of UCB of neonates born to anemic and non anemic mothers

Parameters	Cut off maternal Hb Value 12 gms%		p – Value p < 0.05	Cut offmaternal Hb value 10.9 gms%		p – Value p < 0.05
	Anemic mothers (51) (Mean ±SD)	Non-anemic mothers(49) (Mean ±SD)		Anemic mothers (36) (Mean±SD)	Non-anemic mothers (64) (Mean±SD)	
RBC COUNT/ μl	4.32±0.55	4.19±0.41	0.29	4.39±0.53	4.21±0.49	0.137
PCV %	46.93±6.10	45.87±3.97	0.402	47.33±5.75	46.17±5.27	0.374
Hb gms/dl	15.04±1.93	14.65±1.21	0.33	15.13±1.89	14.79±1.62	0.405
MCV fl	108.74±5.04	109.41±5.20	0.574	107.73±5.29	109.59±4.91	0.126
MCH pg	34.87±1.54	34.96±1.49	0.806	34.47±1.71	35.11±1.39	0.08
MCHC%	32.13±0.91	31.96±0.72	0.384	32.01±0.88	32.10±0.84	0.64

RDW%	18.11±1.74	17.87±1.31	0.521	18.01±2.10	18.04±1.30	0.947
Reticulocyte count %	4.09±0.94	3.65±0.94	0.047	4.30±0.99	3.75±0.90	0.016
Absolute reticulocyte count./µl	17.70±4.64	15.29±4.17	0.023	18.86±4.66	15.85±4.27	0.005
Corrected reticulocyte count.%	4.57±1.17	3.97±1.06	0.026	4.82±1.14	4.13±1.12	0.012
Reticulocyte production index.	4.46±1.33	3.88±1.10	0.05	4.71±1.26	4.03±1.24	0.024
NRBC % (automation)	5.03±10.79	5.36±5.84	0.882	5.81±14.53	4.83±5.29	0.66
NRBC/100 WBC (manual)	8.39±8.30	9.55±12.27	0.616	8.39±8.30	9.55±12.27	0.134
NRBC/µl(automation)	0.62±0.99	0.86±1.09	0.322	0.65±01.25	0.73±0.90	0.72

Table 2: Comparison of RBC parameters of UCB of neonates born to anemic mothers with biological reference interval

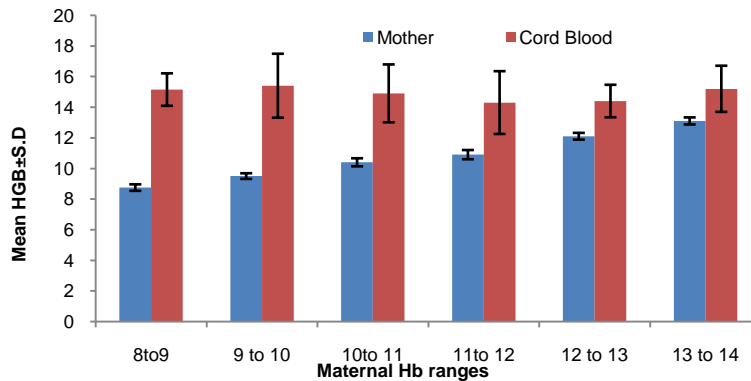| Parameters	Present study	Biological reference interval	P–value
RBC count/ µl	4.19±0.41	4.1±0.4	0.792
PCV %	45.87±3.97	44.6±5.3	0.461
Hb gm%	14.65±1.21	14.9±1.7	0.356
MCV fl	109.41±5.20	108.1±4.8	0.521
MCH pg	34.96±1.49	36±1.7	0.001
MCHC %	31.96±0.72	33.3±0.8	0.001
RDW%	17.87±1.31	18.23±1.35	0.814
NRBC count% (Cut off Hb ≤ 12 gms % in mother)	5.03± 10.79	3.24±3.07	0.172
NRBC count% (Cut off Hb ≤ 10.9 gms % in mother)	5.81±14.53	3.24±3.07	0.132

Figure 1: Comparison of maternal Hb and UCB Hb

DISCUSSION

As the fetus is dependent on maternal blood, whether the anemic conditions of mother reflect on the fetus is being studied in detail. Our results showed no significant variation in the mean values for RBC parameters like RBC count, Hb, packed cell volume(PCV), mean corpuscular volume(MCV), MCH, MCHC and Red cell distribution width(RDW) in the UCB of neonates born to anemic and non anemic mothers. Elgari MM. et al observed the same findings in a study from Saudi Arabian 2013[8]. The reason for maternal anemia not causing neonatal anemia may be due to high iron transfer from mother to fetus. This is explained by earlier studies, which had shown higher cord blood values for ferritin, Hb and transferrtin saturation. Also maximally stimulated erythropoiesis had been noted at the end of gestation by higher values for soluble transferrin receptor and ferritin index[9]. However, when we compared the results of RBC parameters of UCB of neonates born to anemic mothers with our reference range established in 2014, significant low values of MCH and MCHC were found when the mothers were anemic despite the fact that RBC, Hb value and MCV did not show significant difference. This might have been due to early hemoglobin deficiency within the red cell which would not have been reflected in the total Hb value due to high RBC count and other factors. Jasim M et al had also stated lower MCH and MCHC values in maternal anemia[10]. A significant positive Pearson correlation was found between maternal and fetal Hb is in comparison with earlier studies. This explains a dependence of foetal hemoglobin level on certain maternal factors like ferritin and hepcidin[4]. The reticulocyte parameters namely reticulocyte count, absolute reticulocyte count corrected reticulocyte count, reticulocyte production index were significantly increased in the UCB of neonates born to anemic mothers when compared to that of non anemic mothers in agreement with the study on reticulocytes counts done at Saudi Arabia in 2013[8] Elevated reticulocyte production index implicates a hyper erythropoietin state in the intrauterine

period. This state may be due to compensatory mechanism because of low oxygen delivery to the fetus. Though the NRBC count in the UCB of neonates born to anemic women with Hb of less than 10.9gms% was higher, there is no significant elevation. Chronic hypoxic state in the fetus leads to increased erythropoietin and its effects like release of RBC precursors in the UCB and neonates. A high number of intrauterine and post natal factors cause increased erythropoiesis like fetal insulin in diabetic mothers, maternal smoking, prematurity, preeclampsia and anemia[11,12,13]. Since we excluded mothers with these variables and included only full term neonates with normal apgar score, the variability caused by asphyxia was eliminated. It had been reported, increased NRBCs in the neonates is an indication of chronic intrauterine hypoxia[5]. Sidappa AM *et al* in his review article had stated that anemic mothers with mean Hb of 8.72 gms% and increased erythropoietin levels had infants with more active erythropoietic effect secondary to hypoxia in fetus of anemic mothers.[14] Though the reticulocyte parameters in UCB were significantly increased in maternal anemia, our study did not show any increase in NRBC count. This may be due to bone marrow response to erythropoietin releasing reticulocytes as the anemic condition is mild. The NRBCs might have increased if the maternal anemia had been more severe.

CONCLUSION

This study implies that high iron transfer from mothers to fetus and maximum erythropoiesis during term occur to prevent neonatal anemia, as the maternal Hb value reflects the cord blood Hb value. Hence it is essential to prevent maternal anemia and maintain a higher Hb level. Increased NRBCs in the UCB indicates increased risk and more extensive care to the neonate. As each milliliter of blood drawn from infant causes anemia, studies on UCB can be considered a worthwhile alternative to neonatal blood. Also maternal and UCB must be screened for RBC parameters before submitting UCB for costly investigations, storage and stem cell transplants.

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REFERENCES

1. Carroll PD, Nankervis CA, Iams J, Kelleher K. Umbilical cord blood as a replacement source for admission complete blood count in premature infants. J Perinatol. 2012 ;32(2):97-102.
2. Ugwu N, Ugwuja E. Complete venous blood counts in healthy term Nigerian neonates.Journ Biomed Inv. 2009;5(2):57-62.
3. Bari S, Seah KK, Poon Z, Cheung AM, Fan X, Ong SY, et al. Expansion and Homing of Umbilical Cord Blood Hematopoietic Stem and Progenitor Cells for Clinical Transplantation. Biol Blood Marrow Transplant. 2014; 30:1-12.
4. Dapper DV, Didia BC. Haemorheological parameters of umbilical cord blood of Nigerian newborns: correlations with maternal parameters. West Afr J Med. 2006;25(3):226-30.
5. Nordstrom L, Arulkumaran S. Intrapartum fetal hypoxia and biochemical Markers: A review Obst and Gynec Survi 1998; 53: 645-657.
6. Dacie JV and Lewis SM. Hemoglobin Estimation. In: Practical Hematology 11edn. London: Churchill Livingstone Medical Division of Pearson Professional Ltd; 2014; 15-18
7. Suman DF, Raj SS, Priyathersini N, et al. Reference range for hematological profile of umbilical blood: A study conducted at a tertiary care centre in South India (in press)
8. Elgari MM, Waggiallah HA. Assessment of hematological parameters of neonatal cord blood in anemic and non-anemic mothers. J Clin Exp Res 2013;1(2):22-25.
9. Lorenz L,Peter A, Poets C F, Franz A R. A Review of Cord Blood Concentrations of Iron status Parameters to Define Reference Ranges for Preterm Infants. Neonatology 2013; 104:194–202.
10. Al-Marzoki J M,Al-Maaroof Z W, Kadhum A H. Determination of reference ranges for full blood count parameters in neonatal cord plasma in Hilla, Babil, IraqJournal of Blood Medicine 2012:3 113–118.
11. Widness JA, Susa JB, Garcia JF, Singer DB, Sehgal P, Oh W, Schwartz R, Schwartz HC.Increased erythropoiesis and elevated erythropoietin in infants born to diabetic mothers and in hyperinsulinemic rhesus fetuses. J Clin Invest. 1981; 67:637-42.
12. Meberg A,Haga P,Sande H.et al.Smoking during pregnancy- haemotologic observations in the new born. Acta paediatr.Scand.1997; 68:731-734.
13. Darkhaneh RF, Ghanbari A, Asgharnia M, Kian M, Comparision of nucleated red blood cells in the umbilical cord of term neonates in healthy women and women with preeclampsia. Iran J Reported Med 2013; 11:25-30.
14. Siddappa AM, Rao R, Long JD, Widness JA, Georgieff MK. The assessment of newborn iron stores at birth: a review of the literature and standards for ferritin concentrations. Neonatology. 2007;92(2):73-82