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Original Research Article

Comparison of cord blood nucleated red blood cells with clinical markers of perinatal asphyxia

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ABSTRACT

Background: The fetal compensatory response to hypoxia is erythropoiesis resulting in influx of immature red blood cells into fetal circulation, level of which may be correlated with perinatal asphyxia. Nucleated red blood cells (NRBCs) are commonly seen in the circulation of newborns. The number of NRBCs per 100 white blood cells (WBCs) varies and it is usually less than 10. >10 NRBCs are usually seen in cases of prematurity, Rh sensitization, maternal diabetes mellitus and intra uterine growth retardation. Asphyxia is also said to cause an increase in the nucleated RBC'S in the newborns.
Methods: Study included NRBCs/100 white blood cells in umbilical venous sample and it's comparson with clinical markers of perinatal outcome. A prospective study was done between November 2020 to 2022. Subjects included singleton term babies.
Results: The presence of thick Meconium stained amniotic fluid, non-reassuring FHR pattern and low Apgar at 1 minute was associated with higher number of NRBC and was statistically significant.
Conclusions: Nucleated RBCs in cord blood of the newborns are the best correlators of perinatal outcome compared to the clinical markers.

Keywords: Nucleated RBC, Asphyxia, Meconium stained liquor, Apgar score

INTRODUCTION

Perinatal asphyxia is one of the important factors determining the perinatal outcome. Perintal asphyxia is defined as clinical or biochemical evidence of lack of O_2 or excess of CO_2 in the body due to failure of efficient pulmonary respiration at birth with resultant hypoxia and acidaemia.[1] Perinatal asphyxia is one of the leading causes of death in newborns with about 19% of neonatal deaths.[2]

Perinatal asphyxia is defined as failure to initiate or sustain breathing at birth.[3]

Perinatal asphyxia could be either due to a prolonged antepartum hypoxia of the fetus or an acute antepartum compromise.[4]

Perinatal asphyxia is known to complicate approximately 2.2% of all deliveries in a tertiary care unit of developed countries although overall incidence may be upto 5-10%. Reported incidence of cerebral palsy and mental retardation following fetal asphyxia is about 8%.[1,5]

Commonly used clinical parameters of perinatal outcome are: APGAR score, presence of meconium-stained liquor, and intrapartum electronic fetal heart rate monitoring.

METHODS

The study included nucleated red blood cells (NRBCs)/100 white blood cells in umbilical venous sample and its comparison was conducted with clinical markers of perinatal outcome.

Study design

It was a prospective type of study.

Subjects

Singleton term babies born at Sri Ramachandra Medical College and Hospital were the subjects.

Study period

The duration of the study was from November 2020 to November 2022.

Sample size

The sample size was 200 (with sensitivity of 89% and specificity of 100% and precision of 2%).

Inclusion criteria

Patients with term pregnancy (≥37 weeks), women with non-reassuring fetal heart rate pattern, women with meconium-stained liquor, and babies with low Apgar score in ≤6 at 1 minutes were included.

Exclusion criteria

Pregnancies known to be associated with following were excluded: women with Rh isoimmunisation, women with gestational diabetes mellitus, post and pre term pregnancy, small for gestational age, and non-cephalic presentation.

Methodology

From all subjects, samples of cord blood collected immediately after clamping and cutting the umbilical cord.

Sample was taken in EDTA coated bottle for purpose of making smears. Sample should be processed immediately. In case of any delay between the time of collection of sample and the timing of taking the reading, the samples were refrigerated.

A thin smear was made of the umbilical venous blood and stained with Leishmans's stain. The smear was studied under 45x magnification and number of nucleated red blood cells/100 white blood cells was determined by scanning the film from one end till 100 WBC's were counted. The nucleated RBC count/100WBCs of cord blood was determined.

Interpretation

Interpretation included: normal term babies 0-10 NRBC/100 WBC, and asphyxiated babies >11 NRBC/100WBC. Depending upon the severity of asphyxia the number of NRBC/100 WBC increases.

Statistical methods

Descriptive statistics was done for all data and the suitable statistical tests of comparison were done. Clinical markers of perinatal outcome and nucleated RBCs were considered as primary explanatory variables. Descriptive analysis was performed using frequency and proportion for categorical variables. Continuous variables were analysed with unpaired t-test and the association between the categorical variables were analysed with Chi-square test. P values less than 0.05 were considered statistically significant. The final sample size was n=260. CoGuide software was used for the data analysis - BDSS corp, released 2020, coGuide statistics software, version 1.0, India: BDSS corp.

RESULTS

In this study, out of 260 babies, 40 babies had elevated levels of nucleated RBCs (>11/100 WBCs) and 35 babies with presence of clinical markers and all the babies with elevated nucleated RBCs had NICU admission or observation (Table 1).

According to traditional standards, the correlation between age and nucleated RBCs is not statistically significant because $p>0.05$. Age does not vary between groups because it is not statistically significant. In other words, the participants in the groups share the same demographic traits (Table 2).

According to traditional standards, the correlation between gravida and birth asphyxia is not statistically significant because $p>0.05$ (Table 3).

According to traditional standards, the correlation between the colour of the liquor and the nucleated RBCs is statistically significant since $p<0.05$ (Table 4).

The presence of thick MSL had a higher NRBC count and the difference was statistically significant when compared to clear or thin MSL (Table 5).

By conventional criteria the association between the nucleated RBCs and APGAR score at 1 minute is considered to be statistically significant since $p<0.05$. There is real risk of developing poor perinatal outcome if the APGAR scores at 1 minute is significantly low (Table 6).

By conventional criteria the association between the nucleated RBCs and fetal heart rate pattern is statistically significant since $p<0.05$ (Table 7).

Nucleated RBC count (>11 NRBC/100 WBCs) was significantly higher in those with presence of clinical markers (Table 8).

Table 1: Outcome of the study population.

Variables	Clinical markers of perinatal outcome + (%)	Clinical markers of perinatal outcome – (%)	Chi square	P value
Nucleated RBCs 0-10/ 100WBCs (n=220)	1 (0.45)	219 (99.55)	214.98	<0.001
Nucleated RBCs >11/100 WBCs (n=40)	35 (87.5)	5 (12.5)		

Table 2: Age distribution in the study group.

Age (years)	Nucleated RBC <10/100 WBCs (%)	Nucleated RBC >11/100 WBCs (%)	Chi square	P value
≤20	10 (4.54)	4 (10.0)	1.461	0.065
21-30	160 (72.7)	22 (55.0)		
31-40	50 (22.7)	14 (35.0)		

Table 3: Gravidity distribution in study population.

Gravida	Nucleated RBC <10/100 WBC (%)	Nucleated RBC>11/100 WBCs (%)	Chi square	P value
Primigravida	120 (54.5)	17 (42.5)	1.970	0.160
Multigravida	100 (45.45)	23 (57.5)		

Table 4: Colour of the liquor and nucleated RBCs.

Colour of the liquor (n=260)	Nucleated RBC <10/100 WBCs (%)	Nucleated RBC>11/100 WBCs (%)	Chi square	P value
Clear	210 (95.4)	10 (25)	129.060	<0.001
Meconium-stained liquor	10 (4.54)	30 (75.0)		

Table 5: Mean nucleated RBCs in meconium stained liquor.

Mean NRBC± SD in grade 1 MSL (thin MSL)	Mean NRBC±SD in grade 2 and 3 MSL (thick MSL)	P value
10.76±7.9	14.65±7.09	0.01

Table 6: Apgar score at 1 minute and nucleated RBCs.

Apgar score at 1 minute	Nucleated RBC<10/100 WBCs (%)	Nucleated RBC>11/100 WBCs (%)	Chi square	P value
≤6	1 (0.5)	9 (22.5)	45.89	<0.001
>6	219 (99.5)	31 (77.5)		

Table 7: Nucleated RBC count and FHR pattern.

FHR pattern	Nucleated RBC<10/100 WBCs (%)	Nucleated RBC>11/100 WBCs (%)	Chi square	P value
Non-reassuring	1 (0.5)	10 (25.0)	50.328	<0.001
Reassuring	219 (99.5)	30 (75.0)		

Table 8: Comparison of nucleated RBCs and clinical markers of perinatal outcome.

Nucleated RBC/100 WBC	Clinical markers of perinatal outcome present (%)	Clinical markers of perinatal outcome absent (%)	Chi square	P value
<10	10 (24)	210 (97.7)	166.10	<0.001
11-20	11 (22)	4 (1.90)		

Continued.

Nucleated RBC/100 WBC	Clinical markers of perinatal outcome present (%)	Clinical markers of perinatal outcome absent (%)	Chi square	P value
>20	24 (48)	1 (0.47)		

The present study provided the evidence that higher number of nucleated RBCS/100 WBCs were seen in the umbilical cord venous sample of the newborns with clinical markers of perinatal asphyxia. Higher amounts of nucleated RBCS/100WBCs in umbilical cord venous sample were associated with poor early neonatal outcome (NICU admission). Nucleated red blood cell count was found be an effective marker of perinatal outcome.

DISCUSSION

The process of normal labour is perhaps the most difficult times the fetus goes through. The disturbances in uteroplacental circulation during labour and tendency to undergo acidosis have been recognized.[5] Various investigators have proposed that the intrauterine hypoxia caused to the fetus can set off a number of biochemical and hematological responses. It has been observed that hypoxia causes a stimulation of hematopoietic system as evidenced by rise in levels of erythropoietin.[6] This rise in erythropoietin levels translates to the release of erythroid precursors into the circulation of the fetus.

Based on the hypothesis that hypoxia triggers the hematopoietic response this study was aimed to analyze the NRBCs counts in relation to the clinical markers of perinatal asphyxia. There were no cases with diabetes, Rh incompatibility, post-dated pregnancy as these conditions is known to result in raised NRBC counts.[7] Based on the hypothesis that hypoxia triggers the hematopoietic response this study was aimed to analyse the NRBCs counts in relation to the clinical markers of perinatal asphyxia.[8]

There were no cases with diabetes, Rh incompatibility, post-dated pregnancy as these conditions are known to result in raised NRBCs.[9] Nucleated RBC are seen in cord blood of healthy newborns at birth. The number varies between 0-10.[10]

Table 9: Normal levels of NRBCS in various studies.

Studies (year)	NRBCs/100 WBCs
Hanlom–Lundberg et al (1999)[5]	8.55±10
Kirby et al (1999)[15]	8.5±16.2
Cunningham et al (1997)[16]	7.56±3.8
Ghosh et al (2003)[17]	8.6±7.7
Our study	7.6±3.65

Nucleated RBCs and meconium staining of the amniotic fluid

In the cases with meconium-stained amniotic fluid it was found that the mean NRBC count was significantly higher in thick MSAF than in cases with clear liquor or grade 1 MSL.[11,12]

Table 10: Levels of NRBCs in relation to meconium-stained liquor in various studies.

Study (year)	Thick MSL	Clear or thin MSL
Hanlom–Lundberg et al (1999)[5]	11.12±13.77	7.84±8.96
Kirby et al (1999)[15]	12.0±26.4	8.5±15.0
Our study	14.6±7.05	7.65±4.49

In our study elevated NRBCs significantly correlated with presence of thick meconium- stained amniotic fluid and counts were similar to previous studies.

Limitations

The limitation of the study is that the study population and sample size is small and is not a randomized control trial.

CONCLUSION

The quality of amniotic fluid, FHR pattern, APGAR score and cord blood nucleated RBCs count per 100 WBCs was assessed. The presence of thick meconium-stained liquor, non-reassuring fetal heart rate and APGAR <6 in 1 minute showed higher number of NRBCS per 100 WBCs and was statistically significant. Thus, the nucleated RBCs in cord blood of the newborns are good correlator of perinatal outcome.

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