ORIGINAL RESEARCH PAPER

Obstetrics & Gynecology

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A STUDY ON CORD BLOOD NUCLEATED RBC'S AS A MARKER OF FETAL ASPHYXIA

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ABSTRACT

BACKGROUND AND OBJECTIVES: The objective of this study is an attempt to establish a relationship between the levels of nucleated RBC's and to assess the severity of perinatal asphyxia and early neonatal outcome. There by preventing complications such as hypoxic ischemic encephalopathy, neurological impairment and polycythemia.

METHODS: In this study 320 patients who have undergone emergency LSCS at Govt Kilpauk medical college were taken. Singleton term pregnancies primi /multi babies of more than 2.5kg appropriate for gestational age irrespective of indication, without any maternal co morbid factors were taken up.

Inclusion and exclusion criteria, study protocol were designed. Various parameters were also studied including NICU Admissions, relation with gravida, maternal age, LSCS indication, and duration of labour.

RESULTS: NRBC'S were significantly high in cord blood of patients with prolonged first and second stage of labour and who underwent emergency lscs for fetal

distress and deep transverse arrest. Also increased NRBC'S were noted in babies with low apgar score. Babies with birth asphyxia who were diagnosed by the pediatrician, showed an increased levels of NRBC'S. NRBC count increased proportionately to the severity of HIE.

CONCLUSION: From this study it was concluded that estimating the number of nucleated RBC/100 WBC in umbilical cord venous blood sample of new born is an important test, the sample being obtained non invasively from otherwise discarded specimen and analyzed by personnel or equipment readily available in most hospital laboratories. The level of nucleated RBCs/100 WBCs correlates with acute intrapartum asphyxia and can be used as an index of early neonatal outcome

AIM:

1. To determine normal levels of nucleated red blood cells /100 white blood cells in cord blood smear of non asphyxiated term new borns.
2. To establish a relationship between the levels of nucleated red blood cells /100 white blood cells and to assess the severity of perinatal asphyxia

INTRODUCTION

Science has allowed medicine to penetrate the hidden World of the fetus and to begin diagnosis and treat fetal conditions. To obstetricians, the fetus is "the patient within the patient", and part of the discipline of Obstetrics is the care of the fetus[13].

Fetal and neonatal death- leading cause world over is perinatal asphyxia. Perinatal asphyxia can be defined as clinical or biochemical evidence of decrease of oxygen and an increase of carbondioxide in the body because of the deficient respiratory function at birth with resultant hypoxia and acidemia[27]. In the developed countries the percentage of perinatal asphyxia is 2% as shown by LOW, 1998, but the overall percentage is around 5-10%. Cerebral palsy and mental retardation is reported in 8% cases following fetal asphyxia as shown by Blair and Stanley, 1988. The obstetrician has a responsibility in recognizing the hypoxic event so that one can prevent associated morbidity and mortality[14.46].

NRBCs are commonly seen in the circulation of newborns. The number of NRBCs per 100 WBCs varies and it is usually less than 10. Conditions were there is >10 NRBC's are usually seen are 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.[34]

The present study was done to evaluate the significance of presence of nucleated red blood cells/100 white blood cells in a blood smear made from umbilical cord blood (venous) sample. Therefore the aim of this study is to correlate the NRBC levels and acidemia in neonates. The present study was carried out on 320 pregnant women admitted to the labour ward at Govt. Kilpauk medical college and hospital, Chennai.

AIM AND OBJECTIVES OF THE STUDY:

1. To determine normal levels of nucleated red blood cells /100 white blood cells in cord blood smear of non-asphyxiated term new borns.
2. To establish a relationship between the levels of nucleated red blood cells /100 white blood cells and to assess the severity of perinatal asphyxia .
3. To assess the short term neonatal outcome in asphyxiated babies. [perinatal period 1 wk]
4. To correlate NRBC's count with the neonatal outcome associated with perinatal asphyxia. [NICU admissions, HIE& its severity].

MATERIALS AND METHODS:

This study was done in Govtkilpauk medical college, Chennai .Around 320 patients who have undergone emergency LSCS irrespective of indication have been taken to know NRBC's (nucleated redblood cells /100WBC's) as a indicator of perinatal asphyxia.

STUDY DESIGN: Prospective Cross sectional study PERIOD OF STUDY: April 2014 to September 2014

STUDY GROUP: Singleton term pregnancies primi /multi babies of more than 2.5kg appropriate for gestational age delivered by emergency LSCS irrespective of indication without any maternal comorbid factors.

INCLUSION CRITERIA:
Singleton Term Pregnancies
- Primi /Multi
- Babies of more than 2.5kg
- Appropriate for gestational age
- Emergency LSCS

EXCLUSION CRITERIA:
Pregnancies known to be associated with Women with Rh isoimmunization
- Women with gestational diabetes mellitus
- Post term pregnancy
- IUGR
- Pre eclampsia patients
- Newborn with congenital anomalies
- Preterm babies

SAMPLE SIZE: 320 Sample size was determined on the basis of a pilot study in which the incidence of Birth Asphyxia was measured as 28%. We calculated a minimum sample size of 310, assuming a type 1 error (two-tailed) of 0.05 and a margin of error of 5%.

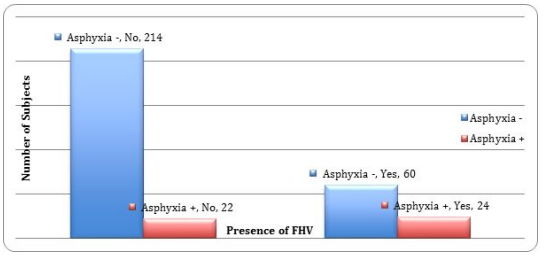
METHODOLOGY:
Sample taken in EDTA coated bottle for purpose of making smears. 2 ml of blood was collected For making smear, two clear glass slides were taken and a drop of sample was placed towards one end. A spreader glass slide placed at 450 inclination to sample and in one uniform motion drop of blood smeared on rest of slide. Slide is allowed to dry and then covered with Leishman's stain. After 5 minutes stain is diluted with distilled water and mixed on slide. Slide is allowed to take in stain for 15 minutes and then washed in gentle stream of water. Under pathologist's supervision, smear focused under high power microscope and RBCs (nucleated) counted against 100 WBCs. 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 of cord blood was determined

RESULTS:
STATISTICS:
Descriptive statistics was done for all data and suitable statistical tests of comparison were done. Continuous variables were analysed with the unpaired t-test and categorical variables were analysed with the Chi-Square Test and Fisher Exact Test. Statistical significance was taken as $P < 0.05$. The data was analysed using EpiInfo software (7.1.0.6 version; Center for disease control, USA) and Microsoft Excel 2010.

Treatment Groups	Name of Group	Treatment	Number of Subjects
Group A	Asphyxia -	Singleton term pregnanciesprimi /multi babies of more than 2.5kg appropriate to gestational age delivered by emergency LSCS irrespective of indication without any maternal co morbid factors without Birth Asphyxia	274
Group B	Asphyxia +	Singleton term pregnanciesprimi /multi babies of more than 2.5kg appropriate to gestational age delivered by emergency lscs irrespective of indication without any maternal co morbid factors with Birth Asphyxia	46

In this study out of 320 babies 46 babies have diagnosed by the paediatrician as birth asphyxia got admitted in NICU and remaining 274 babies were diagnosed as no asphyxia transferred to mothers side .

Fetal Heart Rate Variability

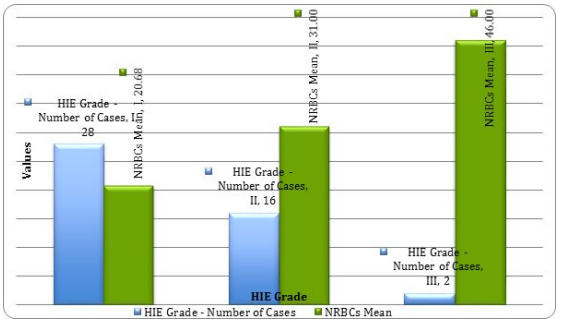


We conclude that there is real intrapartum obstetric risk factor for developing Birth asphyxia if there is presence of foetal heart rate variability in our study. It can be used to predict early birth asphyxia.

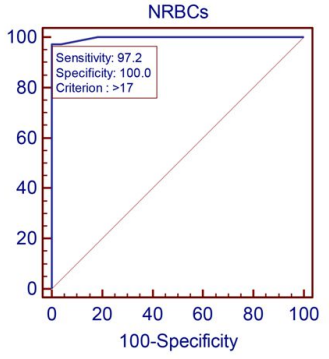
NRBCs
TABLE: Tabulation showing relation between NRBCS and ASPHYXIA

NRBCs	0 to 10	%	11 to 20	%	>20	%
NO ASPHYXIA	224	99.56	50	80.65	0	0.00
ASPHYXIA +	1	0.44	12	19.35	33	100
TOTAL	225	100	62	100	33	100

HIE Vs NRBCs



HIE Grade	No of cases	NRBCs Mean±SD
I	28	20.68±7.32
II	16	31.00±5.93
III	2	46.00±0.00



ROC curve

Variable	NRBCs
Classification variable	HIE_grade HIE grade

Sample size		320
Positive group :	HIE grade = 1	36
Negative group :	HIE grade = 0	284

Disease prevalence (%)	unknown

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	0.996919
Standard Error[a]	0.00311
95% Confidence interval[b]	0.982788 to 0.999925
z statistic	159.857
Significance level P (Area=0.5)	<0.0001

[a] DeLong et al., 1988
[b] Binomial exact

Youden index

Youden index J	0.9722
Associated criterion	>17

Criterion values and coordinates of the ROC curve

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
≥2	100.00	90.3 - 100.0	0.00	0.0 - 1.3	1.00	
>11	100.00	90.3 - 100.0	81.69	76.7 - 86.0	5.46	0.00
>12	97.22	85.5 - 99.9	96.13	93.2 - 98.1	25.10	0.029
>17	97.22	85.5 - 99.9	100.00	98.7 - 100.0		0.028
>46	0.00	0.0 - 9.7	100.00	98.7 - 100.0		1.00

ROC curve shows high sensitivity to detect birth asphyxia by nucleated RBC's .

DISCUSSION:

In the present study, the number of nucleated red blood cells/100 white blood cells in the asphyxia group is high . The incidence of birth asphyxia is more when the NRBCs levels are significantly elevated. It is statistically significant with a p-value of 0.000 according to unpaired t-test. The NRBCs level increased meaningfully more in the Asphyxia+ group compared to No Asphyxia group by 17.33 NRBCs per 100 WBC. The occurrence of birth asphyxia was meaningfully more (97.83%).when the NRBCs level increased more than 10 NRBCs per 100 WBC

SUMMARY:

1. In the present study cord blood have been collected from 320 patients Singleton term pregnancies primi /multi babies of more than 2.5kg appropriate for gestational age delivered by emergency lscs irrespective of indication without any maternal co morbid factors.

We conclude that increased NRBCs levels correlates well with development Birth asphyxia. Hence NRBC levels can be a useful for the evaluation of perinatal asphyxia where facilities of pH sampling are not available and can serve as a reliable, inexpensive and easily available marker of perinatal asphyxia. [13].Neonate with Nucleated RBC'S more than 10 was admitted to NICU and had poor neonatal outcome. Estimation of NRBCs may be an easy and simple investigation and may be used as an indicator of fetal asphyxia in the future.

CONCLUSION:

To conclude estimating the number of nucleated RBC/100 WBC in umbilical cord venous blood sample of new born is an important test, the sample being obtained non invasively from otherwise discarded specimen and analyzed by personnel or equipment readily available in most hospital laboratories. The level of nucleated RBCs/100 WBCs correlates with acute intrapartum asphyxia and can be used as an index of early neonatal outcome.

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