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A Prospective Study -Evaluation of Nucleated Red Blood Cells Pattern Of Normal Newborn With That Of The Asphyxiated Newborn

[1]Dr. Deepa Dharanappa, [2] Dr. Akash Gadgade , [3]Dr. Kavita Yevoor,[4] Dr. Priya Patil, [*]Dr. Lata D Shetty

[*]Professor, [1,4]Assistant professor, [2,3]Associate Professor,

[*]Department of Anaesthesia, Mahadevappa Rampure Medical College. Kalaburgi , Karnataka , India

[1]Department of Pediatrics,Malla Reddy Medical College of Women, Hyderabad, India

[2] Department of Pharmacology,Srinivas Institute of Medical sciences,Mangalore,India

[3]Department of Pathology,KIMS,Hubli,India

[4]Department of Pathology, BIMS, Belagavi,India

***Corresponding Author:**
Dr. Lata D Shetty
Dr. R R Shah Hospital, MSK Mill Road, Opp Govt ITI College, Kalaburgi, Karnataka, 585102

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Abstract

Objectives: To compare nucleated red blood cells pattern of normal newborn with that of the asphyxiated newborn and to determine the relationship between the nucleated RBCs counts. Design: Prospective study. Setting: Tertiary Neonatal Intensive Care Unit.

Methodology: Forty asphyxiated newborns and forty non-asphyxiated neonates (control) were the sample of the study. Term appropriate for gestational age with Apgar score of <7 at 1 and/ or 5 minutes were included in the study. Blood sample from specified asphyxiated newborns and control group were subjected for nucleated RBC count along with other, hematological parameters.

Results: In the 80 neonates studied, there was not much variation in routine hematological parameters. Nucleated RBC count was significantly higher in the asphyxiated newborns (10±4.34 and range 3-22 nRBC/ 100 WBCs) as compared to non-asphyxiated newborns (4.37±3.16 and range 1-12 nRBC/ 100 WBCs) (p-value <0.001). Mortality was 12.5% among asphyxiated newborns.

Conclusion: As providers of perinatal care, we seek to determine markers of asphyxia, nucleated RBC count may be such a marker. It is highly specific, quick, reliable, cost effective, does not require specific expertise and hi-tech institutional facilities, hence, it can be used as a marker of asphyxia and predictor of immediate neurological outcome.

Keywords: Nucleated red blood cells; asphyxiated newborn; nucleated RBC count; Hematological parameters; Birth asphyxia, Neonatal brain injury

Introduction

Birth asphyxia is a serious problem worldwide, resulting in nearly one million deaths and an equal number with neurologic sequelae annually.[1] Neonatal brain injury due to intrapartum asphyxia is an important cause of cerebral palsy, mental retardation and epilepsy. Despite advances in perinatal care over the past three decades, the incidence of cerebral palsy attributed to birth asphyxia has not changed. One reason is that we do not know specifically how to intervene postnatally to prevent hypoxic ischemic encephalopathy (HIE), which may follow intrapartum asphyxia and ultimately result in cerebral palsy. We also do not know how to identify the neonates with asphyxia who are at greater risk for encephalopathy and therefore are most likely to benefit from an intervention.

Perinatal hypoxic ischemic cerebral injury begins during an asphyxial insult, usually caused by an interruption in placental blood flow and gas exchange and extends into a recovery period after resuscitation. It is during this interval after resuscitation from hypoxia ischemia that an intervention to reduce the severity of ongoing brain damage might be efficacious. One might anticipate that the presence of acidosis in the umbilical cord arterial blood would be a good measure of the severity or duration of intrauterine asphyxia and correlate with outcome. However, most term infants with severe acidemia at the time of delivery have an uncomplicated neonatal course. Thus, it is not surprising that the common factors assessed during labour to identify fetuses with asphyxia that are at risk for brain injury, such as fetal heart rate (FHR) abnormalities and thick meconium staining of amniotic fluid, low Apgar score rarely predict subsequent cerebral palsy.

Nucleated RBC (nRBC) count may be one marker for intrauterine hypoxia contributing to adverse neonatal outcomes. In the presence of a hypoxic insult, fetal erythropoietin is released and stimulates the early release of red blood cells from the bone marrow to increase haematocrit and increase the oxygen carrying capacity of the blood. Hence, more of nRBCs are released in the peripheral blood. Therefore, elevated nRBC counts have been suggested both as a marker of acute and chronic intrauterine fetal hypoxia and as a predictor of adverse neonatal outcome. Therefore, we conducted this study to evaluate the absolute nucleated red blood cell count in asphyxiated babies and its correlation with the neurological outcome.

Methodology:

40 asphyxiated neonates born or admitted in NICU in BLDEA's Medical college Hospital and Research centre, Bijapur and 40 the neonates born at BLDEA's Medical college Hospital and Research centre, Bijapur were taken as cases and controls respectively. The demographic profile and relevant information of individual patient was collected through a pre-designed and pre-tested structured questionnaire format by interviewing the mother and examination of individual newborn.

The inclusion criteria for cases was term, appropriate for gestational age neonates with APGAR score of less than 7 at 1 and/ or 5 minutes and for controls, term, appropriate for gestational age with APGAR score 7 at 1 and 5 minutes. Rh typing and ABO incompatibility, infants of diabetic mothers, twins, preterm, intrauterine growth retardation neonates were excluded from the study. If immediately after birth, the baby was asphyxiated, resuscitation was done as per standard protocol. Apgar score were noted during resuscitation. If at 1 and/ or 5 min the score was <7, peripheral venous blood was collected within 6 hours of birth into an ethylene diaminetetracetic acid (EDTA) tube and a smear was made. The smear was stained with Leishman stain and number of nucleated red blood cells per 100 WBC was calculated. Nucleated RBC was expressed as the number per 100 WBCs. The blood samples collected were also analyzed for hemoglobin, total and differential leukocyte count.

During hospital stay, the babies were followed up till discharge or death and different events were recorded. The staging of hypoxic ischemic encephalopathy was done according to Sarnat and Sarnat staging. During the same study period blood samples of normal newborn which act as control was taken and subjected to similar haematologic evaluation. These babies were regularly followed up or examined until discharge, and were neurologically normal until discharge. Statistical tests of significance (students 't' test) were applied and the predictive values (sensitivity, specificity, PPV, NPV) were calculated using the conventional formulae.

Results:

The study consisted of 40 asphyxiated and 40 non-asphyxiated neonates as control. Among the non-asphyxiated babies, 19(47.50%) were males and 21(52.50%) were females.

Table 1: Parameter distribution among the study population

Parameter	Non-asphyxiated babies	Asphyxiated babies

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	No. of Cases	**Percentage**	**No. of Cases**	**Percentage**
Sex distribution				
Male	19	47.50	25	62.50
Female	21	52.50	15	37.50
Birth order distribution				
First	16	40.00	24	60.00
Second	17	42.50	12	30.00
□3rd	7	17.50	4	10.00
Mode of delivery				
NVD	27	67.50	24	60.00
LSCS	13	32.50	16	40.00
Birth weight				
2500 – 3000	32	80.00	33	82.50
3001 – 3500	8	20.00	6	15.00
3501 – 4000	0	0	1	2.50

Among the asphyxiated babies, 25(62.50%) were males and 15 (37.50%) were females. In the control, 67.5% babies were delivered by normal vaginal route and other 32.5% were delivered by LSCS.

In the asphyxiated 60% babies were delivered by normal vaginal route and 40% by LSCS. Among the controls the maximum 17(42.5%) babies were second order and least i.e., 7 (17.5%) were □3rd order. Among the cases the maximum 24 (60%) babies were first order and least i.e., 4 (10%) were □3rd order.

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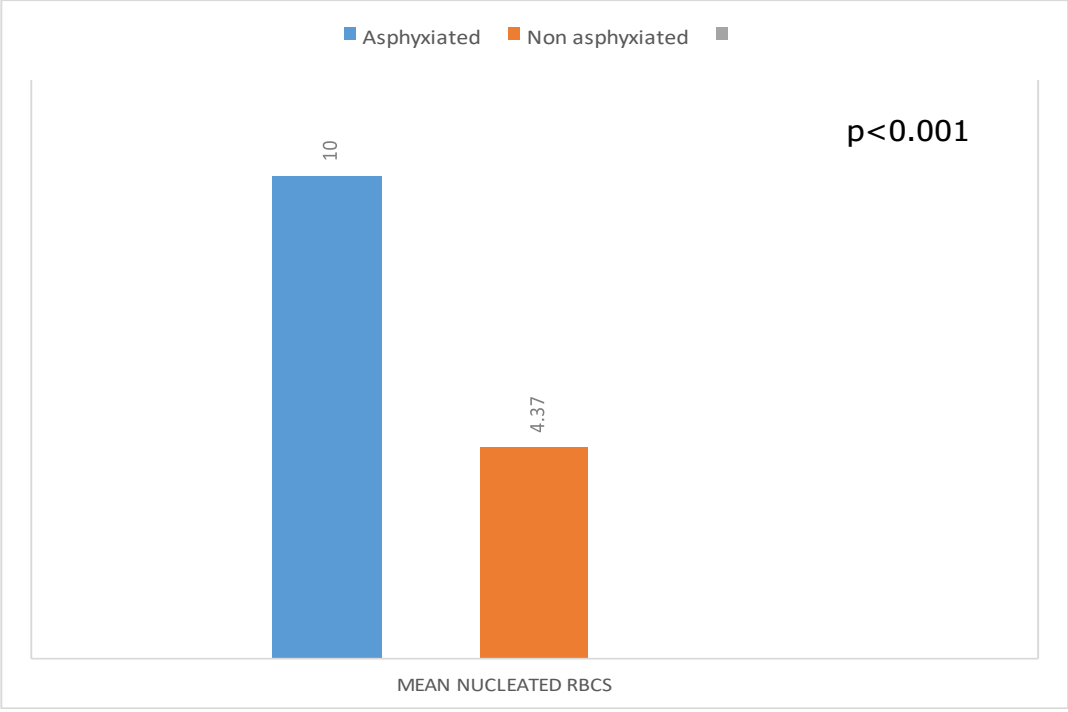
Among the controls maximum (80%) babies weighed between 2500-3000 gm and no babies weighed between 3501-4000 grams. In the asphyxiated group 33 (82.5%) babies weighed between 2500-3000 grams and 1 (2.5%) baby weighed between 3501-4000 grams.

Table 2: Nucleated RBCs/ 100 WBC in asphyxiated and non-asphyxiated neonates (control)

nRBCs/100 WBC	Asphyxiated group	Non-asphyxiated group	P-value
0 – 5	6	29	
6 – 10	18	8	
11 – 15	12	3	
16 – 20	3	0	
21 – 25	1	0	
26 – 30	0	0	
Mean±SD	10±4.34	4.37±3.16	<0.001
Range	3 – 22	1 – 12	

Fig. 1.: Mean Nucleated RBCs/ 100 WBC in asphyxiated and non-asphyxiated neonates (control)



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The asphyxiated group had a significantly higher number of nucleated RBC (mean±SD 10±4.34 and range 3-22 nRBCs/100 WBCs), when compared to the non-asphyxiated group (mean±SD 4.37±3.16 and range 1-12 nRBCs/ 100 WBCs) and this observation was highly significant statistically ($p<0.001$).

After the above observation, 10 was taken as a cutoff value between normal and asphyxiated group, and they were grouped as >10 and □10. It was noticed that 16 cases were true positive and 3 cases were false positive with respect to value of >10. With cutoff value of □10 cases, 24 cases were false negative and 37 were true negative.

Discussion:

Nucleated red blood cells (nRBC) are commonly seen on the first day of life in the cord blood and peripheral blood of most newborns. In the normal newborn, the number of nRBCs is dependent on the gestational age of the fetus i.e., with advancing gestational age, there is decline in the nRBCs.[8,12,13]In the term non-asphyxiated infant, the number of nRBCs is variable but is rarely higher than 10.[7,8,12] Naeye and Localio[14] were the first who reported the concept of neonatal nRBC as potential markers for timing of hypoxic insult in November 1999. Other similar studies done by Phelan et al[7] and Korst et al[8] also concluded that nRBCs are a potential marker of fetal asphyxia. Our results in non-asphyxiated term neonates are in keeping with these observations. Blackwell, Sean et al also, in their study concluded that mean nucleated RBC counts for neonates with early-onset seizures were significantly increased compared with those of control neonates[3].

Previous studies have suggested that nRBCs increase in response to an asphyxiation event.[2,11,12] As observed in our study, the nRBC count was significantly higher in the "asphyxiated" group. The time required to produce a rise in nRBCs count is unknown but appears to be relatively short in light of the rapidity of response observed in the uterine rupture group in a study done by Phelan et al.[7]

In the absence of an asphyxial event, pre-existing IUGR,[13] maternal diabetes mellitus,[12] or prematurity[12] could produce an elevated nRBC count.[9]

Neonatal brain injury due to intrapartum asphyxia is an important cause of cerebral palsy, mental retardation and epilepsy. Fetal erythropoietin (EpO) production is clearly regulated by requirements for tissue oxygenation, however, elevated EpO concentrations (up to 8000 mU/mL) have been reported in several pathologic states, such as fetal hypoxia, anemia (e.g., caused by fetal Rhesus hemolytic disease), placental insufficiency or infants of diabetic mothers[9].Despite advances in perinatal care over the past three decades, the incidence of cerebral palsy attributed to birth asphyxia has not changed. One reason is that we do not know specifically how to intervene postnatally to prevent hypoxic ischemic encephalopathy, which may follow intrapartum asphyxia and ultimately results in cerebral palsy. Nor do we know how to identify the neonates with asphyxia who are at greatest risk for encephalopathy and therefore are most likely to benefit from an intervention.

Male-female ratio in controls in the present study was 0.9:1 and among cases was 1.7:1. The majority of deliveries in our study among controls and cases were by normal vaginal delivery, and maximum number of mothers were primiparous (60%) in asphyxiated group, followed by 2nd and □3rd order. Term babies >2.5 Kg were included. Among non-asphyxiated group, maximum number of babies i.e., 80% were between 2500-3000 grams. Among asphyxiated group, maximum number of babies (82.5%) were also between 2500-3000 grams followed by 3001-3500 grams and 3501-400 grams. However, there was no significant variation with respect to various hematological parameters. In a study by Thilaganathan B et al[6], the relationship of umbilical cord blood arterial pH, Apgar score[16], leukocyte count and erythroblast count at delivery in term neonates born vaginally and by elective cesarean section and by emergency cesarean section for abnormal intrapartum FHR pattern (55 infants) was studied. This study suggested that leucocytosis is a non-specific response of the fetus to labor, whereas increase in erythroblast count reflects fetal tissue hypoxia.

Nucleated red blood cell counts are a potentially useful tool in estimating the degree and timing of intrauterine hypoxia[2]. Nucleated RBCs are a common observation in the circulating blood of newborn infants.[8] The number of nRBCs/ 100 WBCs is quite variable but is rarely >10. In those instances, in which the number of nRBCs exceeds 10, the most frequent explanations for the increase are prematurity[12], rhesus sensitization[13], maternal diabetes mellitus[11] and intrauterine growth

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restriction,[13] and in our study, all these conditions have been excluded through exclusion criteria to improve the validity of our study. Asphyxia has been suggested to induce a rise in the number of nucleated RBC in the newborn[7].However, in a study done by Hamrick SE et al[4] concluded that in a population of neonates with perinatal depression, the nRBC count at birth does not correlate with magnetic resonance spectroscopy or 30 month neurodevelopmental outcome. This study suggests that the nucleated RBC count should not be used as a surrogate marker for subsequent brain injury.

In the present study, the non-asphyxiated neonates i.e., control group had a mean±SD of 4.37±3.16 nRBC range of 1-12 nRBC for 100 WBC, the asphyxiated group had a mean±SD of 10±4.34 nRBC and a range of 3-22 nRBC for 100 WBC and it had a p-value of <0.001. Similar observations were made by Phelan JP et al[7] and Korst LM et al[8]. Buonocore G et al in his study concluded that increase in nucleated RBC count at birth not only reflects a response of the infant to perinatal hypoxia but is also a reliable index of perinatal brain damage[5].

As providers of perinatal care, we continually seek to determine markers of intrauterine hypoxia, which contribute to adverse neonatal outcome. Nucleated red blood cell count may be one such marker. Birth asphyxia occurs worldwide and is one of the major cause of morbidity and mortality in the newborn, its incidence and associated sequelae are high. In our study of asphyxiated neonates and their correlation to nucleated RBC count, exhibited significantly high number of nucleated RBCs than non-asphyxiated group. This simple laboratory test is easy to perform, quick, highly specific and cost effective. Moreover, attempts at prevention of the brain injury caused by intrauterine asphyxia, antepartum and intrapartum, demands precise awareness of when such injury is imminent[15].

With these observations, we conclude that the nucleated RBC count in a new born is of high predictive value in fetal asphyxiation and its outcome and it has specific value.

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