Umbilical Cord Blood Nucleated Red Cell Count as A Marker of Perinatal Asphyxia: A Case Control Study

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Abstract

Background: Perinatal asphyxia is one of the leading causes of neonatal mortality and morbidity worldwide, accounting for a significant proportion of deaths and long-term neurological impairments. Present study aimed to evaluate the correlation between Nucleated RBC and APGAR scoring. **Material and Methods:** A total of 150 newborns were included from Department of Pathology at the Adesh Institute of Medical Sciences and Research in Bathinda, of which 100 were classified as cases— meconium-stained amniotic fluid (MSAF) in newborns. Neonates given clear amniotic fluid made up the control group. Newborns with MSAF were classified as cases, while those with clear fluid were placed in the control group. APGAR score was recorded. Samples were drawn into EDTA-coated vacutainers and analyzed using an automatic cell counter. To evaluate the entire haematological profile, blood smears were made. Nucleated red blood cells (NRBCs) and reticulocyte count was done. **Results:** The mean maternal age and gestational age was slightly higher in the case group compared to the control group, though this difference was not statistically significant. The case group's birth weight was significantly lower (2570 ± 570 g) than the controls' (2840 ± 410 g), and this difference was highly significant (p = 0.001). Mean hemoglobin levels, mean WBC count and Platelet counts was not statistically significant. In contrast, the mean NRBC/100 WBC and absolute NRBC count was highly significant. Significant negative correlation was found in cases with Apgar score and NRBCs value stating that as Apgar scores decreased the NRBCs increased. **Conclusion:** Study shows positive results with elevated NRBC counts, particularly when combined with low Apgar scores, can serve as a reliable and inexpensive marker for identifying neonates at risk of perinatal asphyxia in MSAF cases.

Keywords: Perinatal asphyxia, haematological profile, Nucleated red blood cells.

INTRODUCTION

Meconium, the newborn's first stool, when mixed with amniotic fluid (meconium-stained amniotic fluid, MSAF) and is linked to fetal hypoxia.[1] Perinatal asphyxia—oxygen deprivation before, during, or after birth—affects 5–10% of deliveries and is one of the main causes of newborn morbidity and mortality.[2] Diagnosis relies on multiple parameters such as Apgar score, cord blood pH, and fetal monitoring, but no single marker is fully reliable.

Nucleated red blood cells (NRBCs) is normally rare in older children but common in newborns leading to increase in response to hypoxia due to elevated erythropoietin levels, causing their release from bone marrow.[3] Several studies show that high NRBC counts in cord blood correlate with intrauterine hypoxia and perinatal asphyxia. Many diagnostic tests are costly and limited to advanced centers. NRBC counting is inexpensive and feasible even in rural primary health centers. Present study aims to evaluate effects of meconium stained liquor on Nucleated RBC's and reticulocyte count in cord blood and to compare NRBC Count in relation to APGAR score.

MATERIALS AND METHODS

Present observational study was conducted in the department of Pathology at the Adesh Institute of Medical Sciences and Research in Bathinda, for a period of 2 years. A total of 150 newborns were included, of which 100 were classified as cases— meconium-stained amniotic fluid (MSAF) in newborns. Neonates given clear amniotic fluid made up the control group. Comprehensive maternal histories were obtained, and mothers with conditions such as diabetes, hypertension, preeclampsia, COPD, or a history of smoking during pregnancy were excluded. Details of labour and delivery were recorded. Newborns with MSAF were classified as cases, while those with clear fluid were

placed in the control group. After the membranes ruptured, the amniotic fluid was tested for the presence or lack of meconium. APGAR score was recorded. From each newborn, two millilitres of cord blood were taken under aseptic conditions right after the umbilical cord has been clamped and severed. Samples were drawn into EDTA-coated vacutainers and analyzed using an automatic cell counter. To evaluate the entire haematological profile, blood smears were made. Nucleated red blood cells (NRBCs) and reticulocyte count was done.

RESULTS

For the study, one fifty newborns were included. Records were kept of the mean maternal age, gestational age, parity, and delivery method.

[Table 1] presents a comparison of demographic and perinatal characteristics between the case group (neonates born with meconium-stained amniotic fluid) and the control group (neonates with clear amniotic fluid). The mean maternal age was slightly higher in the case group (25.35 ± 4.50 years) compared to the control group (24.50 ± 3.00 years), though this difference was statistically significant (p = 0.009). Gestational age was also higher in the case group (38.60 ± 1.40 weeks) than in controls (38.10 ± 1.20 weeks) with a significant p-value of 0.036. Mean parity was marginally higher in cases (2.00 ± 0.85) than controls (1.85 ± 0.65), but without statistical significance. The case group's birth weight was significantly lower (2570 ± 570 g) than the

controls' (2840 ± 410 g), and this difference was highly significant (p = 0.001).

[Table 2] compares the hematological profiles of the case group (MSAF) and the control group. Mean hemoglobin levels were slightly higher in cases (14.90 ± 2.20 g/dL) compared to controls (14.55 ± 1.75 g/dL), but this difference was not statistically significant (p = 0.395). The mean WBC count was elevated in the case group ($13,600.45 \pm 4,890.20$ /cumm) relative to the control group ($12,150.32 \pm 3,420.18$ /cumm), though the difference did not reach statistical significance (p = 0.096). Platelet counts were marginally lower in cases (2.72 ± 0.92 lakh/mm³) than in controls (2.88 ± 0.82 lakh/mm³), also without significant difference (p = 0.162). In contrast, the mean NRBC/100 WBC was markedly higher in the case group (11.10 ± 4.80) compared to controls (3.25 ± 1.70), with a highly significant p-value (p = 0.0002). Similarly, the absolute NRBC count was substantially elevated in cases ($1,720.40 \pm 1,390.50$ /cumm) versus controls (400.12 ± 260.34 /cumm), again with a highly significant p-value (p = 0.0002). These findings indicate that while general hematological parameters such as hemoglobin, WBC, and platelet counts were similar between groups, NRBC counts were significantly higher in neonates with MSAF, suggesting their potential role as a marker for fetal hypoxia.

[Table 3 & 4] shows the coefficient correlation linear regression analysis between NRBCs (NRBCs/100 WBC and absolute NRBCs) in case group with Apgar score at 1 and 5 minute. Significant negative correlation was found in cases with Apgar score and NRBCs value stating that as Apgar scores decreased the NRBCs increased.

Table 1: Comparison of Case and Control Groups			
Parameter	**Case group**	**Control group**	**p-value**
Maternal age (yrs)	25.35 ± 4.50	24.50 ± 3.00	0.009
Gestational age (wks)	38.60 ± 1.40	38.10 ± 1.20	0.036
Male/Female	26/22	27/21	1.000
Parity	2.00 ± 0.85	1.85 ± 0.65	0.112
Birth weight (gms)	2570 ± 570	2840 ± 410	0.001
Mode of delivery (ND/CS)	28/20	30/18	0.835

Table 2: Haematological Profile Comparison in the Case and Control Groups			
Hematological Characteristics	**Case Group (Mean ± SD)**	**Control Group (Mean ± SD)**	**p-value**
Hemoglobin (g/dL)	14.90 ± 2.20	14.55 ± 1.75	0.395
WBC Count (/cumm)	$13,600.45 \pm 4,890.20$	$12,150.32 \pm 3,420.18$	0.096
Platelet Count (Lakh/mm³)	2.72 ± 0.92	2.88 ± 0.82	0.162
NRBCs/100 WBCs	11.10 ± 4.80	3.25 ± 1.70	0.0002
Absolute NRBCs (/cumm)	$1,720.40 \pm 1,390.50$	400.12 ± 260.34	0.0002
Reticulocyte count	$4.8+1.6$	$3.4+1$	0.0001

Table 3: Association of APGAR Score (1 & 5 min) of newborns in case and control group						
Parameter	**Study Group**	**Minimum**	**Maximum**	**Mean**	**SD**	**p value**
Apgar Score (1 min)	Control	6	9	8.1	0.74	0.001
	Case	4	8	6.4	1.1	
Apgar Score (5 min)	Control	7	10	8.8	0.43	0.001
	Case	5	9	7.7	1.0	

Mean Apgar score at first minute was 8.1 ± 0.74 in control group whereas in cases group it was 6.4 ± 1.1. Mean Apgar score at five minutes was 8.8 ± 0.43 in control group whereas

in cases group it was 7.7 ± 1.0. Difference of Apgar score (1 and 5 minute) was significant between cases and control group.

Table 4: Relationship between NRBC and APGAR score in cases group				
Variable	**Absolute**	**NRBC/100 WBC**	**APGAR at 1 min.**	**APGAR at 5 min.**

Cases	NRBC r-value	p-value	r-value	p-value	r-value	p-value	r-value	p-value
Absolute NRBC	1.000	-						
NRBC/100 WBC	0.951	0.000	1.000	-				
APGAR at 1 min.	-0.760	0.000	-0.736	0.000	1.000	-		
APGAR at 5 min.	-0.580	0.000	-0.513	0.000	0.697	0.000	1.000	-

DISCUSSION

The study investigated the effect of meconium-stained amniotic fluid (MSAF) on the hematological profile of newborns and compared the findings with several previous studies. Demographic comparisons showed that maternal age in both case and control groups were similar to values reported in earlier research, with minor variations. Gestational age was slightly higher in the case group in the present study, a difference that was statistically significant, and consistent with some studies such as Darkhaneh et al. though others like Boskabadi et al. reported no difference.[2,7] Gender distribution between groups was comparable and was not statistically significant, similar to previous studies. Birth weight was generally lower in the MSAF group than in controls, a trend supported by studies from Tungalag et al and Colaco et al, although other researchers did not find a significant difference.[6,8] In both groups, normal vaginal delivery was the most common mode of delivery, followed by cesarean section, in line with findings from Colaco et al. and Tungalag et al.[6,8]

Hematological profile analysis revealed that hemoglobin, total WBC count, and platelet counts did not differ significantly between groups, similar to the results from Brucknerova et al. and Darkhaneh et al.[2,5] However, some authors, such as Colaco et al, reported significantly higher WBC counts in MSAF cases.[6] Platelet counts were comparable in the present study and in some previous work, though Boskabadi et al. observed a marked reduction in platelets in their case group.[7]

A key finding of the current study was the significantly elevated nucleated red blood cell (NRBC) counts—both per 100 WBC and absolute counts—in the MSAF group compared to controls. This difference is consistent with numerous studies (e.g., Tungalag et al., Bayati et al., Darkhaneh et al., Hassan Boskabadi et al., Colaco et al.), all of which reported higher NRBC counts in cases, supporting the role of NRBCs as a marker of intrauterine hypoxia.[2,4,7,8] The variability in NRBC counts across cases was partly explained by differences in labor duration, MSAF thickness, and possible absence of hypoxic insult in some newborns.[9-11] Additionally, the mean reticulocyte counts in cases (4.8%) were substantially greater than those in controls (3.4%), and a strong positive correlation was observed. While literature on reticulocyte counts in this context is limited, studies such as Rawat et al and Sarcoglu et al. have linked increased reticulocyte levels to hypoxic or hemolytic conditions, supporting the present study's findings. Apgar scores at both 1 and 5 minutes were significantly lower in MSAF cases compared to controls, and there was a significant inverse relationship between Apgar scores and NRBC counts (r = –

0.736 at 1 min; r = –0.513 at 5 min). This inverse relationship was in agreement with reports from Colaco et al, Tungalag et al, Boyati et al. and others, who observed that higher NRBC counts are associated with lower Apgar scores and greater degrees of birth asphyxia.[6,8,4]

CONCLUSION

Overall, the study's findings align closely with the broader literature, reinforcing that elevated NRBC counts, particularly when combined with low Apgar scores, can serve as a reliable and inexpensive marker for identifying neonates at risk of perinatal asphyxia in MSAF cases.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Shashikala A, Raghavan S. Perinatal Outcome in relation to mode of delivery in MSAF. Indian J of Pediatrics 1995; 62:63-67.
2. Darkhaneh RF, Asgharnia M, Yousefi TZ. Comparison of NRBC in term neonatal umbilical cord blood between neonates with meconium stained amniotic fluid and clear amniotic fluid. J Turkish-German Gynecol Assoc 2008;9(2):29-31.
3. Aziz M, Ali M, Hakim S.Evaluation of hematological profile of cord blood and placental histopathology in neonates with perinatal asphyxia. CurrPediatr Res 2012; 16 (2):105-110.
4. Bayati M, Abid M, Wahbi A. Role of nucleated red blood cells in umbilical cord blood as a marker of neonatal asphyxia with meconium stained amniotic fluid. The Iraqi post graduate medical journal 2014; 13(2):336-341.
5. Brucknerova I, Ujhazy E, Dubovicky M, Mach M. Early assessment of the severity of asphyxia in term newborns using parameters of blood count. InterdiscToxicol 2008; 1:211-213.
6. Colaco M, Ahmed M, Bajpai R. Study of nucleated red blood cell counts in asphyxiated newborns and the fetal outcome. Int J ClinPediatr2014; 3:79-85.
7. Boskabadi H, Maamouri G, Sadeghian H et al. Early diagnosis of perinatal asphyxia by nucleated red blood cell count. Archives of Iranian medicine 2010;13:275-281.
8. Tungalag L, Gerelma G. Nucleated Red Blood Cell Counts in Asphyxiated Newborns. Open Science Journal of Clinical Medicine 2014; 2(1):33-38.
9. Phelan JP, Ahn MO, Korst LM et al. Nucleated red blood cells-A marker for fetal asphyxia. Am J ObstetGynecol1995; 173: 1380-4.
10. Abhishek MG ,Sarvesh BM. Study of association between perinatal asphyxia and nucleated red blood cell count in umbilical cord blood. International journal of advances in case reports 2015; 2:1003-1006.
11. Geetika, Shweta Sikarwar, Gupta Sunita. The Correlation of Clinical Perinatal Asphyxia with Counts of Nrbc/100 Wbc in Cord Blood. Webmed Central Obstetrics and Gynecology. 2011; 2(1):1-9.