Medical Laboratory Technology Journal
9(1), 2023, 63-69
Received 2023-28-03; Revised 2023-06-05; Accepted 2023-10-05
Available online at : http://ejurnal-analiskesehatan.web.id

Haematological Parameters in Preterm Neonates Admitted in Neonatal Intensive Care Unit in a Tertiary Care Hospital

Dwepa Kamlesh Parikh, *Amit Ravindra Nisal, Ravindra Chandrashekhar Nimbargi

Department of Pathology, Bharati Vidyapeeth (Deemed To Be) University Medical College and Hospital, Pune, Maharashtra, India.
*Email: dramitnisal@gmail.com
DOI: 10.31964/mltj.v8i2.517

Abstract: Though reference haematological parameters are defined for neonates, ranges vary in preterm. Few data are available regarding the premature population during the first month of life. Objective: To observe the variation in haematological parameters concerning different gestational ages, birth weights and gender among preterm neonates admitted to NICU with common illnesses like respiratory distress (RDS), neonatal hyperbilirubinemia (NNH) and sepsis in a tertiary care hospital. A total of ninety preterm neonates admitted to the neonatal intensive care unit (NICU) were analyzed over two years. Complete blood counts were obtained, grouped and analyzed according to the underlying diagnosis of sepsis, NNH and RDS. Clinical data were also extracted. The data were analyzed using SPSS software version 25. Mean, Chi-square test and ANOVA tests were used for data analysis. P value <0.05 was considered significant. Result: Variation was seen concerning gestational age and birth weight. Eosinophils were significantly decreased in LBW, while a decrease in neutrophils and an increase in lymphocyte count were seen in EPT. Haemoglobin and RBC indices also showed significant variation according to birth weight. Conclusions research complete blood counts of preterm depend on the degree of prematurity, birth weight, and other clinical findings.
Keywords: Birthweight; neonatal hyperbilirubinemia; prematurity; sepsis

INTRODUCTION

According to the WHO, preterm birth is the birth of a live child before the mother has reached 37 full weeks of pregnancy. The preterm infants are grouped as per gestational age into 1) Extremely preterm (EPT)- <28 weeks (195 days), 2) Very preterm (VPT)- <32 weeks (237 days), 3) Moderate preterm (MPT)- Between 32 to <34 weeks and 4) Late preterm (LPT)- Between 34 to <37 weeks of gestation (238 to 258 days) while infant classification by birth weight is classified as: Normal birth weight (NBW)- from 2,500 to 4,000 grams and Low birth weight (LBW)- less than 2,500 grams. LBW infants can be further sub-classified as low birth weight (VLBW)- less than 1,500 grams and extremely low birth weight (ELBW)- less than 1,000 grams (Effer SB et al., 1969).

Numerous studies have been done on the typical changes in reference paediatric blood parameters during the first few years of life. These physiological variations in term newborns have been studied at birth, in the first few hours and days after birth, and up to six months. Preterm neonates have less complete physiological data but are more likely to need transfusions or anti-infective treatments that are adjusted based on blood counts (Roudil P et al., 2017).

Corresponding Author: Amit Ravindra Nisal
Department of Pathology, Bharati Vidyapeeth (Deemed To Be) University Medical College and Hospital, Dhankawadi, Pune- 411043. Maharashtra. India.
Email: dramitnisal@gmail.com

The variation in parameters among preterm compared to term neonates is not extensively studied, and it is unknown how preterm infants' hematologic profiles differ in relation to typical prematurity-related complications (Roudil P et al., 2017). Research is needed, besides studying various parameters according to gestational age and birth weight, also studying changes in haematological parameters in premature neonates with sepsis, RDS and NNH, which have not been extensively analyzed in other studies. Therefore, this study analysed the spectrum of haemograms of preterm neonates admitted to the NICU. To evaluate and stratify them according to preterm type and to determine the association between CBC parameters with respect to clinical findings in preterm infants like sepsis, neonatal hyperbilirubinemia (NNH) and respiratory distress syndrome (RDS).

MATERIALS AND METHODS

This cross-sectional observational study was conducted for two years, from 1st July 2020 to 30th June 2022, at the Department of Pathology in a tertiary care hospital attached to a private medical college in western India. A total of ninety preterm neonates born between 26 to less than 37 weeks of gestation and admitted to NICU were analyzed. Detailed clinical history of the neonates (including birth weight, gestational age, and presenting complaints) was extracted. This study was approved by the institutional ethics committee.

The peripheral blood samples were collected in a K_2EDTA vacutainer for Complete Blood Counts (CBC) on day 0 of the preterm. CBC was performed on a fully automated Beckman Coulter DxH800 Haematology analyzer. Peripheral blood smears stained with Leishman stain were examined. Reticulocyte count was manually done by New Methylene blue staining procedure and expressed as % of reticulocyte count after examining under a microscope (Shaffer M et al., 1963). There were no anticipated risk factors involved in this study except those occurring due to the natural process of disease progression.

The data were analyzed using SPSS (Statistical Package for social sciences) version 25.0 software. The results were presented in tabular and graphical format. Chi-square and ANOVA tests were used to find the association between two or more attributes for qualitative data variables; for Quantitative data Mean ± SD was calculated. An unpaired t-test was used to compare the two independent groups for Quantitative data variables. A P-value of < 0.05 was considered significant. This study was approved by the institutional ethics committee. (Institutional Ethical Committee Ref No.- BVDUMC/IEC/135).

RESULTS AND DISCUSSION

A total of ninety preterm neonates were included in the present study, with a mean gestational age of 32.06 ± 2.29 weeks and an average birth weight of 1527.22 ± 540.09 grams. There were 41 female babies and 49 male babies. The haematological parameters included the levels of haemoglobin, total leucocyte count (TLC), neutrophils %, lymphocytes %, eosinophils %, monocytes %, platelet count and RBC indices, etc.

Eosinophils were found to be significantly decreased in LBW as compared to NBW neonates (Table 1), while a decrease in neutrophils and an increase in lymphocyte count was seen in EPT as compared to LPT neonates (Table 4). Septic neonates showed variations in morphological abnormalities like leucopenia, toxic changes and shift to the left (Figure 1), which was statistically significant for gestational age.

In contrast to sepsis, neonates with RDS showed a significant decrease in MCV and MCH and an increase in eosinophil counts in NBW compared to ELBW infants. There was a significant increase in haemoglobin, eosinophils, RBC count and hematocrit in LPT as compared to EPT neonates in these neonates.

The comparison of the distribution of neonates with NNH based on the type of birth weight and gender did not differ significantly (P value= 0.067 as per ANOVA test), as shown in Table 2. Also, the distribution of neonates with NNH on the basis of type of preterm birth (gestational age) and gender did not differ significantly (P value= 0.186 as per ANOVA test), as shown in Table 3. We found four cases of ABO incompatibility showing the presence of spherocytes and polychromasia on PBS (Figure 2). Neonates with NNH showed a significant decrease in MCV and MCH in NBW compared to ELBW neonates. Also, a significant increase in haemoglobin, RBC count and hematocrit were seen, and a significant decrease in eosinophil counts, MCV and polychromatic cells in LPT compared to EPT infants in these cases.

Table 1. Comparison of Haematological Parameters According to Birth Weight

Birth	**ELBW (n=20)**		**LBW (n=33)**		**NBW (n=12)**		**VLBW (n=25)**		**P**
weight	Mean	SD	Mean	SD	Mean	SD	Mean	SD	**Value**
Hemoglobin	17.07	3.24	17.07	2.77	16.56	2.83	16.79	2.63	0.942
TLC	9871.2	4548.6	13008.2	5292.31	12010.5	4110.39	10785.0	6380.50	0.177
Neutrophils	64.30	11.29	66.97	7.41	62.67	11.33	65.48	14.63	0.668
Lymphocytes	25.95	10.20	24.33	8.96	27.17	11.05	26.08	15.73	0.883
Eosinophils	1.15	1.69	1.33	1.57	2.42	2.91	0.64	0.81	**0.032***
Monocytes	6.90	2.63	6.76	2.46	7.33	3.65	6.76	3.37	0.944
Platelet Count	183650	55168	219848	80784	222333	79785	188588	80937	0.21
RBC Count	4.58	0.88	4.86	0.77	4.90	0.93	4.74	0.91	0.65
Hematocrit	52.85	10.00	52.18	8.64	51.83	8.82	53.25	7.86	0.956
MCV	115.60	7.62	108.60	6.48	106.38	6.48	111.56	6.51	**0.001***
MCH	37.31	2.35	35.43	2.26	34.45	2.45	35.89	2.33	**0.006***
MCHC	32.29	1.00	32.63	0.74	32.38	1.00	32.17	0.77	0.214
RDW	18.67	1.98	17.42	1.90	18.57	2.67	18.70	4.00	0.244
nRBCs	45.25	72.55	18.33	53.73	18.25	38.85	66.40	203.89	0.436
Reticulocyte Count	5.85	3.62	6.12	4.35	4.42	3.65	5.72	2.87	0.602

*Eosinophils, MCV and MCH showed statistically significant variation according to birth weight (P value <0.05 as per ANOVA test)

Table 2. Comparison of Gender and Type of Birth Weight among Neonates with NNH

Birth weight	**Females**		**Males**		**P**
	N	**%**	**N**	**%**	**value**
ELBW	10	32.3	4	11.1	0.067
VLBW	10	32.3	11	30.6	
LBW	9	29.0	12	33.3	
NBW	2	6.5	9	25.0	
Total	31	100.0	36	100.0	

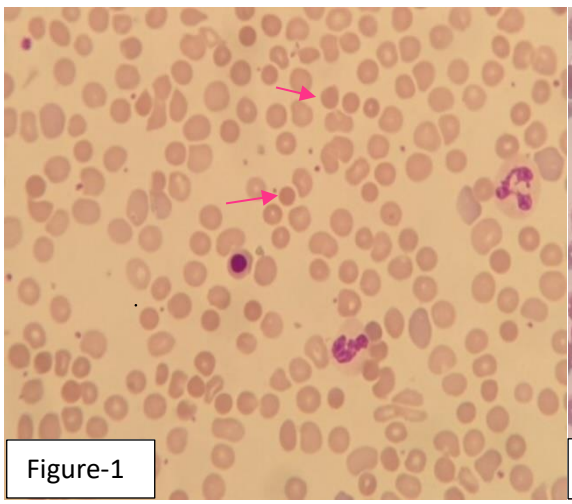
Table 3. Comparison of Gender and Type of Preterm Birth (Gestational Age) among Neonates with NNH

Gestational Age	Females N	%	Males N	%	P value
EPT	3	9.7	2	5.6	0.186
VPT	16	51.6	14	38.9	
MPT	8	25.8	7	19.4	
LPT	4	12.9	13	36.1	
Total	31	100.0	36	100.0	

Table 4. Comparison of Haematological Parameters According to Gestational Age

Birth Weight	EPT (n==8)		VPT (n=36)		MPT (n=22)		LPT (n=24)		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Value
Hemoglobin	14.29	2.96	17.71	2.83	17.06	2.45	16.49	2.57	**0.013***
TLC	10428	6064	10158	4778	11596	6161	14007	4742	0.05
Neutrophils	58.38	17.59	65.50	11.31	67.36	8.94	65.75	9.60	0.271
Lymphocytes	31.25	20.09	26.64	11.65	22.68	9.67	24.67	9.02	0.293
Eosinophils	1.75	1.98	0.69	1.09	1.41	2.17	1.75	1.87	0.087
Monocytes	5.75	2.66	6.89	2.81	7.77	3.21	6.38	2.75	0.261
Platelet count	171750	75388	186638	69669	208122	79909	234958	76367	0.061
RBC Count	3.93	0.96	4.87	0.77	4.87	0.86	4.82	0.81	**0.033***
Hematocrit	44.81	9.89	55.34	8.21	52.91	7.76	50.72	8.07	**0.008***
MCV	114.68	7.59	112.81	7.71	110.53	6.28	106.30	5.64	**0.002***
MCH	36.64	2.48	36.45	2.52	35.88	2.35	34.64	2.11	**0.029***
MCHC	31.96	1.28	32.31	0.73	32.46	0.74	32.59	0.97	0.294
RDW	17.73	1.96	18.91	3.45	18.07	2.11	17.44	2.22	0.219
nRBCs	65.38	104.43	51.67	170.18	30.59	69.65	13.88	33.36	0.581
Reticulocyte count	5.75	3.41	6.22	3.30	5.27	3.74	5.38	4.42	0.762

*Statistically significant variation was seen in Haemoglobin, RBC count, HCT, MCV and MCH with gestational age. (P value <0.05 as per ANOVA test)



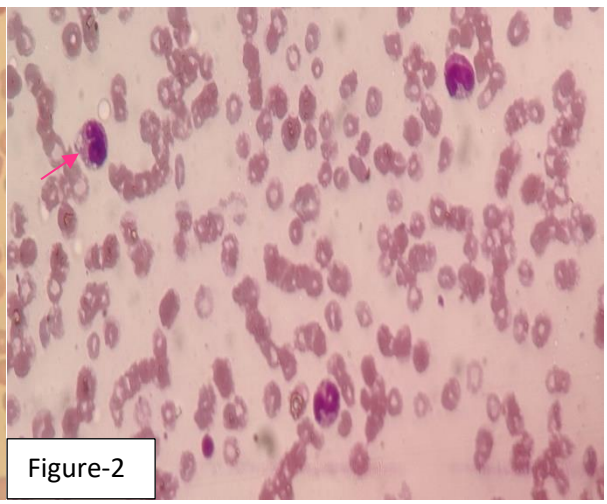


Figure 1. Shows a Peripheral Blood Smear (Leishman stain- 400x magnification) of a Patient with ABO Incompatibility Showing Spherocytes Pointing to the Evidence of Hemolysis.

Figure 2. Shows a Peripheral Blood Smear (Leishman stain- 400x magnification) with the Shift to Left in WBCs (band cells) and Vacuoles in Neutrophils Pointing to the Evidence of Sepsis.

The typical variations in reference paediatric blood parameters during the first few years of life have been the subject of numerous studies. Preterm neonates are more likely to require transfusions or anti-infective treatments adjusted based on blood counts, despite having less complete physiological data (Roudil P et al., 2017; Swaanenburg JC et al., 1987; Taylor M R et al., 1997).

Haematological measurements such as the Haemoglobin (Hb) level, white blood cell (WBC) count, and platelet count are frequently used to assess preterm neonates. It is said that these quick screening tests have good sensitivity and negative predictive values and are widely available (Tigabu Kebede Z et al., 2020). Several authors have noted variations in the hematologic parameters of preterm newborns according to gestational age and birth weight (Roudil P et al., 2017).

The goal of the current study was to evaluate the haematological parameters in preterm neonates admitted to the NICU while concentrating on complications like sepsis, RDS, and NNH. We found an increase in Haemoglobin, RBC count and hematocrit among late preterms compared to extremely preterm neonates with NNH and RDS, which was statistically significant (p-value <0.05), which was also an observation by Rolim et al (Rolim, A. C. B. et al., 2019). As per Roudil et al., all three bloodlines increased proportion to gestational age, which also correlated with our study (Roudil P et al., 2017).

According to Jun-Ho Wu et al., there was an initial trend of a decrease in TLC count and then again an increase after 31 weeks of gestation, along with an increase in haemoglobin levels up to 34 weeks of gestation in Taiwanese preterm infants, which correlated with our study as well. There was no statistically significant change in platelet counts in the study by Jun-Ho Wu et al., which correlates with our study (Wu J. H. et al., 2009). According to Henry, for preterms, leucocytosis is common at birth with a wide reference range. There are increased segmented neutrophils, bands and metamyelocytes, but there is no sign of illness. Preterms have higher absolute neutrophil counts in their neutrophilic leukocytes than older children, who typically have higher lymphocyte counts. The neutrophilic leucocyte count increases within the first twelve hours after birth, drops between one month and one year, and then gradually stabilises at 4.4 10^9/L at around four years of age (Henry E et al., 2015). Yang JY et al. discovered a relationship between birth weight, gestational age and the degree of eosinophilia. Sepsis and RDS were associated with a higher eosinophil percentage, which correlated with our study in the fact that the eosinophil count significantly increased with increasing birth weight in septic neonates and with increasing gestational age in neonates with RDS (Yang J. Y. et al., 2014; Peng CT et al., 1989). Prematurity is a risk factor for neonatal sepsis, which along with anaemia, triggers infantile respiratory distress (Tigabu Kebede Z et al., 2020; Christensen R. D. et al., 2015).

Based on gestational age, Salsbury found that premature infants reach their normal hematocrit sooner and at a lower level than term. (Salsbury D. C. et al., 2001). As per Noguera NI et al., low birth weight newborns had slightly higher hematocrit and RBC count values than the full-term group, while preterms had slightly lower Hb concentrations and higher MCV values (Noguera N. I. et al.,1999). According to Khurana R et al., infants with ABO incompatibility had significantly higher mean reticulocyte counts, which also correlated with our study in the fact that increased polychromasia was seen in extremely preterm neonates with NNH (Khurana R. et al., 2019). Limitation of this research is that studies should be done with a larger sample size to validate the results. It will also help to establish baseline reference ranges for preterm neonates in a geographic population.

CONCLUSION

The present study concludes that certain blood parameters from complete blood count, like Haemoglobin, eosinophils, RBC count, HCT, MCV and MCH, depend on both the degree of prematurity, birth weight and other associated clinical conditions. Awareness regarding these physiological variations may help to take therapeutic decisions in everyday practice.

CONFLICT OF INTEREST

The authors declare that they have no potential conflicts of interest to disclose.

FUNDING

No funding was required for this study.

REFERENCES

Christensen, R. D., Baer, V. L., & Yaish, H. M. (2015). Thrombocytopenia in late preterm and term neonates after perinatal asphyxia. *Transfusion*, *55*(1), 187–196. https://doi.org/10.1111/trf.12777.

Effer SB. (1969). Management of high-risk pregnancy: report of a combined obstetrical and neonatal intensive care unit. *Can Med Assoc J*, 101(7), 55-63. PMID: 5344991; PMCID: PMC1946255.

Henry, E., & Christensen, R. D. (2015). Reference Intervals in Neonatal Hematology. *Clinics in perinatology*, *42*(3), 483–497. https://doi.org/10.1016/j.clp.2015.04.005

Khurana, R., Batra, P., Faridi, M., & Khan, N. (2019). Revisiting ABO incompatibility as a risk factor for significant neonatal hyperbilirubinemia. *Tropical doctor*, *49*(3), 201–204. https://doi.org/10.1177/0049475519838428.

Noguera, N. I., Detarsio, G., Pérez, S. M., Bragós, I. M., Lanza, O., Rodríguez, J. H., Acosta, I., Davoli, R., & Milani, A. C. (1999). Hematologic study of newborn umbilical cord blood. *Medicina*, *59*(5 Pt 1), 446–448.

Peng CT, Lin HC, Wang DW, Tsai CH. (1989). Degenerative changes in neutrophils as an indicator of neonatal sepsis. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi, 30*(5), 309-15.

Rolim, A. C. B., Lambert, M. A., Borges, J. P. G., Abbas, S. A., Bordin, J. O., Langhi Junior, D. M., Chiba, A. K., & Santos, A. M. N. D. (2019). Blood cells profıle ın umbılıcal cord of late preterm and term newborns. *Revista paulista de pediatria: orgao oficial da Sociedade de Pediatria de Sao Paulo*, *37*(3), 264–274. https://doi.org/10.1590/1984-0462/;2019;37;3;00008

Roudil P, Vasselon C, Trombert-Paviot B, Berger C, Patural H. (2017). Blood parameters of preterm neonates: postnatal evolution according to gestational age. *Int J Lab Hematol, 39*(3), 317-328. doi: 10.1111/ijlh.12629. Epub 2017 Apr 19. PMID: 28422440.

Salsbury D. C. (2001). Anemia of prematurity. *Neonatal network : NN*, *20*(5), 13–20. https://doi.org/10.1891/0730-0832.20.5.13.

Shaffer, M., Kiser, G., Luban, N. L., & DePalma, L. (1993). Performance of reticulocyte counts in stored blood specimens in a pediatric population utilizing new methylene blue. *Pediatric pathology*, *13*(5), 591–595. https://doi.org/10.3109/15513819309048247.

Swaanenburg JC, Rutten WP, Holdrinet AC, van Strik R. (1987). The determination of reference values for hematologic parameters using results obtained from patient

populations. *Am J Clin Pathol, 88*(2), 182-91. doi: 10.1093/ajcp/88.2.182. PMID: 3618550.

Taylor, M. R., Holland, C. V., Spencer, R., Jackson, J. F., O'Connor, G. I., & O'Donnell, J. R. (1997). Haematological reference ranges for schoolchildren. *Clinical and laboratory haematology*, *19*(1), 1–15. https://doi.org/10.1046/j.1365-2257.1997.00204.x

Tigabu Kebede, Z., Matebe, Y. H., Demisse, A. G., Yimer, M. A., Mekasha, A., Worku, A., Demtse Gebremedhin, A., McClure, E. M., Nigussie, A. K., Worku, B., Gidi, N. W., Metaferia, G., Goldenberg, R. L., & Muhe, L. M. (2020). Hematologic Profiles of Ethiopian Preterm Infants With Clinical Diagnoses of Early-Onset Sepsis, Perinatal Asphyxia, and Respiratory Distress Syndrome. *Global pediatric health*, *7*, 2333794X20960264. https://doi.org/10.1177/2333794X20960264

Wu, J. H., Chou, H. C., Chen, P. C., Jeng, S. F., Chen, C. Y., Tsao, P. N., Hsieh, C. J., Huang, H. M., & Hsieh, W. S. (2009). Impact of delivery mode and gestational age on haematological parameters in Taiwanese preterm infants. *Journal of paediatrics and child health*, *45*(6), 332–336. https://doi.org/10.1111/j.1440-1754.2009.01497.x

Yang, J. Y., Cha, J., Shim, S. Y., Cho, S. J., & Park, E. A. (2014). The relationship between eosinophilia and bronchopulmonary dysplasia in premature infants at less than 34 weeks' gestation. *Korean journal of pediatrics*, *57*(4), 171–177. https://doi.org/10.3345/kjp.2014.57.4.171