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Original Research Article

Hematological profile including alkali resistant hemoglobin of neonates at birth using cord blood in relation to gestational age and maternal diseases

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ABSTRACT

Background: The importance of complete hemogram along with the hematological indices in the diagnosis of neonatal health cannot be overemphasized. There is a severe paucity of relevant data regarding neonatal hematological profile in relation to gestational age and maternal diseases in this part of the country. With this perspective, the authors intended to do a pilot study to analyse the haematological profile of neonates of different gestational ages and different maternal diseases born in a teriary care hospital in Eastern India.
Methods: This is a cross sectional study design based on cord blood sample of newborns and their mothers recruited from those admitted in the gynae and obstetric department in IPGME&R. The umbilical cord blood was collected from the newborn babies and then examined for different haematological parameters. The data obtained was statistically analysed.
Results: While there has been a sequential rise of fetal red cell count, hemoglobin and the total white cell count neutrophils with gestational age, the mean corpuscular cell volume decreased. All these values were comprised to varying degrees in maternal diseases except for nucleated rell blood cells which conspicuously increased in neonates of diabetic mothers.
Conclusions: In this study, a small attempt was made to assess the haematological profile (including alkali resistant hemoglobin) of the newborns in relation to gestational age and maternal diseases in the population attending a tertiary care hospital in Eastern India.

Keywords: Neonates, Haematological profile, Alkali resistant hemoglobin, Different gestational age, Maternal diseases

INTRODUCTION

Haematopoiesis starts early in the growing embryo and undergoes serial adaptation to meet progressively changing demands for oxygen in embryo, foetus and the neonate through the various stages of foetal and neonatal development. From the 4th month of foetal development, haematopoiesis commences in the bone marrow and at birth it is the sole source of blood cells. Careful examination of a well prepared and well stained blood smears taken from the cord blood in concert with quantification of the blood elements (erythrocytes or red cells, leucocytes or white cells, and thrombocytes or platelets) and evaluation of a variety of hematologic parameters often yields important diagnostic information regarding the neonate at birth. As it is well known that there can be no universal or international standard haematological parameters and that reference values are

heavily influenced by age, race sex, ethnicity, diet and other geographical factors, it is of paramount importance that standard reference levels of haematological parameters of the neonate in the local population should be established.[2,3] But, normative values for the blood elements and the haematologic indices (MCV, MCH, MCHC, etc,) for the term and pre-term neonate are sadly lacking in this part of the country. Though pregnancy is said to be a harmonious coexistence between the foetus and the mother, various maternal diseases like diabetes, pregnancy induced hypertension, anemia all have adverse effect on the haematopoietic system of the foetus. Any relevant data regarding the effect of various maternal disorders on the newborn haematological profile is also lacking in our set up.

The present study was conducted in a tertiary care centre in the eastern part of the country with the aim to assess the normative haematological values in term and preterm neonates from umbilical cord blood. There is enough of evidence to suggest that, umbilical cord blood reflects the health status of the newborn and may be used as an alternative source of newborn blood which may be difficult to obtain. The effects of maternal conditions like diabetes, anaemia and pre-eclampsia on the fetal health are also reflected in the cord blood.[4] In this study, we have also tried to look at the altered haematological profile of neonates of mothers suffering from gestational diabetes, pregnancy induced hypertension and anaemia as seen in the umbilical cord blood.

Aims and objectives

The aim of the study is to determine the normative values of haematological profile along with the haematological indices in the term and the pre-term neonate born to mothers attending a tertiary care hospital in the eastern part of this country. In this study, we have also tried to take a look at the haematological parameters in the newborn of mothers suffering from anaemia, diabetes and pregnancy induced hypertension.

METHODS

The study was conducted in the Department of Pathology and the Department of Gynecology and Obstetrics within the confines of The Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India, one of the teaching institutes of Kolkata, West Bengal, India. A written permission was taken from the Director and also from the Ethical Committee of this Institute. About 200 cases were selected by simple random sampling from the newborn infants delivered either by vaginal or by Cesarean Section in the Department of Obstetrics and Gynecology of the Institute. Infants are included in the study if they were born at the Institute. Infants are excluded in case of birth outside the Institute, any history of antepartum hemorrhage, twin birth, hydrops foetalis, intrauterine transfusion, twin-to-twin transfusion, foeto-maternal hemorrhage, DIC, or any shock like state of the neonate in the immediate post-partum period. In this simple prospective study, detailed history of the mother was taken and physical examination done to note the gestational age and to find any medical and surgical conditions. Antenatal USG was done to determine the gestational age precisely. Routine investigations of blood and urine were done to identify anaemia, diabetes and sugar in urine in the pregnant mothers. After delivery of the baby, the umbilical cord as immediately clamped and 3ml of blood was collected from the umbilical cord by milking in EDTA vials. The blood sample was then transported to the laboratory as soon as possible and analyzed for various haematological parameters within 3-6 hours. A blood smear made and stained with Wright's stain was examined under light microscope using x10, x 40 and oil immersion lenses. Reticulocyte count was done after preparation of a smear stained with new methylene blue. The same blood sample was also analysed in an automated cell counter. After preparation of a haemolysate, alkali resistant haemoglobin was estimated by Singer's method.[5]

The following parameters were studied: hemoglobin and hematocrit, total and differential leukocyte count, platelet count, nucleated RBC and reticulocyte count, red cell distribution width, RBC indices –MCV, MCH, MCHC, and alkali resistant haemoglobin. For each parameter, the accumulated data were grouped for term and preterm infants; mothers with no complication and mothers with complications like anaemia, diabetes and hypertension. The data thus compiled was analyzed by SPSS 16.0 Mean, mode, median of the data were derived. Unpaired T test was done to reveal any significant differences between the groups.

RESULTS

A total of 131 neonates that were included, of these 106 babies were more than 37 weeks, 22 babies were between 32-37 weeks and only 3 babies were less than 32 weeks in their gestational age. Out of the 131 newborn babies, 79 were born of mothers without any complication in the antenatal period. 14 babies were born of anaemic mothers, 16 of pregnancy induced hypertension and 22 of mothers were suffering from gestational diabetes.

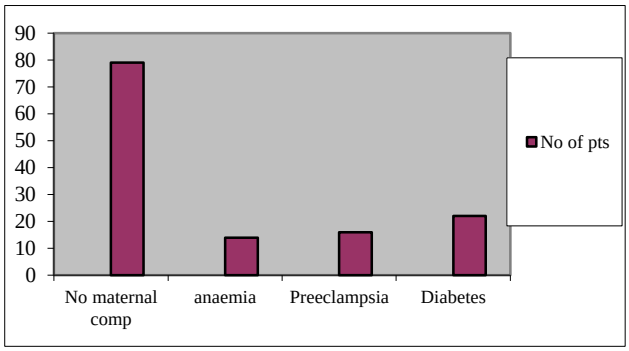

Figure 1: Distribution of babies according to maternal diseases.

The statistical analyses of the compiled data regarding the haematological indices are given in details below.

Hemoglobin

The new born babies were assessed for cord blood hemoglobin. The mean Hemoglobin level in the cord blood of all the newborn babies was 14.87±1.10 (mean±SD) gm/dl and the range was 12.5 g/dl-17.6 g/dl**.** In the present study, the mean and standard deviation of cord blood hemoglobin in full term babies (>37 weeks) who were delivered of mothers without any complications in their antenatal period (n=66) was 16.65±1.15gm/dl and the same for babies of 32-36 weeks gestation was 14.39±0.49 gm/dl and that of babies less than 32 weeks was12.75±0.35gm/dl.

Table 1: Mean and standard deviation of cord blood hemoglobin in all the new born babies.

Range of cord blood hemoglobin in gm/dl	No of new born babies	Mean hemoglobin in gm/dl
16.1gm/dl and above	27	16.58
15.1gm/dl-16.0 gm/dl	23	15.36
14.1gm/dl-15 gm/dl	43	14.6
13.1gm/dl-14 gm/dl	35	13.73
less than 13.0 gm/dl	3	12.76

Table 2: Mean and standard deviation of cord blood hemoglobin in different groups of newborn babies.

	>37 weeks	32-37 weeks	<32 weeks
No Maternal complications	15.27±1.08	13.95±0.51	12.75±0.35
Maternal anaemia	15.05±1.24	13.87±0.66	13.2
preecclampsia	14.82±1.35	14.6±0.48	---
Gestational diabetes	15.02±1.07	---	---

Hematocrit

The mean cord blood hematocrit in all the new born babies was 46.45%±3.5 (mean±SD) and range 38.9%-56.8%.

In the present study the hematocrit of cord blood in term babies (>37 weeks) who were born of mothers without any maternal complications was 47.15±3013% and in babies of 32-36 weeks gestation, it was 43.87±0.59% and in <32weeks babies it was 42.2±0.28%.

RBC count

RBC count in cord blood was measured in all cases.

Table 3: Cord blood hemocrit level in 131 cases.

Range of cord blood hematocrit in %	No of new born babies	mean hematocrit in %
above 50%	26	43.3
45.1%-50%	61	47.4
40%-45%	44	43.3

Table 4: Mean and standard deviation of cord blood hematocrit in different groups of newborn babies.

	>37 weeks	32weeks-37 weeks	<32 weeks
No Maternal complications	47.27±3.13	43.87±0.63	42.2±0.28
Maternal anaemia	51.15±2.03	43.88±1.12	42.1
preecclampsia	46.27±4.97	47.92±0.25	----
Gestational diabetes	46.49±4.37	----	----

Table 5: Mean and standard deviation of cord blood RBC count in different groups of newborn babies.

	>37 weeks	32weeks-37 weeks	<32 weeks
No Maternal complications	4.59±0.29	4.21±0.18	3.75±0.14
Maternal anaemia	4.86±0.32	4.2±0.25	3.8
preecclampsia	4.49±0.44	4.57±0.26	----
Gestational diabetes	4.68±0.48	----	----

Table 6: Mean and standard deviation of cord blood MCV in different groups of newborn babies.

	>37 weeks	32weeks-37 weeks	<32 weeks
No Maternal complications	102.95±4.4 fl	104.28±4.18 fl	114.15±5.12fl
Maternal anaemia	105.34±3.5 fl	104.70±4.33 fl	110.7 fl
preecclampsia	103.82±5.36 fl	104.99±5.57 fl	----
Gestational diabetes	99.46±4.08 fl	----	----

Table 7: Mean and standard deviation of MCH in cord blood of newborn babies.

	>37 Weeks	32-37Weeks	<32Weeks
No Maternal Complication	33.27±4.4	33.15±1.30	34.46±0.36
Maternal anaemia	32.04±3.1	33.76±1.36	34.7
Preeclampsia	33.42±1.9	31.95±1.26	-----
Maternal diabetes	32.21±1.62	-----	-----

Table 8: Mean and standard deviation of MCHC in cord blood of newborn babies.

	>37Weeks	32-37Weeks	<32Weeks
No maternal complication	32.35±1.51	31.80±0.91	30.21±1.04
anaemia	30.4±3.4	32.25±0.54	31.3
preeclampsia	32.13±1.2	30.46±0.94	-----
diabetes	32.41±1.5	-----	------

RBC morphology

In the present study red cell morphology were normocytic and normochromic in all cases.

NRBC count

Nucleated RBC count was performed in all cases in the peripheral blood smear prepared from cord blood of all the new born babies. The mean value was 5.19%±1.7. The value of mean cord blood nRBC count in >37 weeks was 5.2%, it was 4.86% in neonates of 32-36 weeks and 7% in neonates<32 weeks gestation.

The total leukocyte count in cord blood was determined in all the cases. The mean and standard deviation in term neonates (>37 weeks) was 15,886/cmm and in neonates of 32-36 weeks gestation it was 15,894/cmm and in babies <32 weeks gestation it was 18,333/cmm. The mean differential neutrophil count was 65.7% in term newborn babies and the mean differential lymphocyte count in cord blood of term infants was 31% in present study.

The mean differential monocyte and eosinophil count in term newborn babies was 1% and 2.3% respectively in our study. McIntosh had found the corresponding values to be 3% and 6% respectively. The mean total leukocyte count in cord blood in anaemic mothers was 16,178/cmm. The mean fetal hemoglobin considering all infants was 64.6%. In 32-37 weeks gestation it was 67.6%. In <32 weeks gestation it was 85.3%. In anaemic mothers it was 65.38%.

Total and differential leukocyte count

The mean total leukocyte count in cord blood of all the newborns was 15,690/cmm, in babies of 32-37 weeks gestation was 15,894/cmm, and in babies of <32 weeks gestation was 18,333 /cmm. The differential leukocyte count in cord blood of term and preterm babies are as follows:

Table 9: Differential leukocyte count.

	Neutrophil	Lymphocyte	Eosinophil	Monocyte	Basophil
>37 Weeks	65.7	31	2.3	1	---
32-37 Weeks	65.68	29.72	3.5	1.1	----
<32 Weeks	69.9	27	2	1.1	---

Reticulocyte count

Reticulocyte count was performed in all cases from the cord blood of all the new born babies. The mean Reticulocyte count with standard deviation in all newborns was 4.29%±1.1, in babies having gestation age <32 weeks the mean reticulocyte count was 7.5%, in babies of gestational age 32-37 weeks, the mean reticulocyte count was 4.45%.

In the present study nRBC count was performed in all cases in the stained slide prepared from the cord blood. The mean value was 5.19 %±1.7. The value of mean cord blood nRBC count in >37 weeks was 5.2%±1.2, it was 4.86%±1.25 in 32-36 weeks newborns and 7%±1.05 in <32 weeks gestation neonates.

Platelet

In present study the mean platelet count in cord blood in cases of babies with gestation age between 32-36 weeks was 2.42±0.26/cmm and in those with <32 weeks gestation it was 2.55±0.7/cmm. In present study the mean cord blood platelet count in cases of babies born to mothers with pregnancy induced hypertension was 2.14±0.39 lakhs/cmm, in case of >37 weeks gestation 2.33±0.19 lakhs/cmm in case of 32-36 weeks gestation.

Table 10: Platelet count in different groups of neonates.

	>37Weeks	32-37Weeks	<32Weeks
No maternal complication	2.57 ±0.48lakhs/cmm	2.42±0.26lakhs/cmm	2.55±0.7/cmm
anaemia	2.32±0.42lakhs/cmm	2.46±0.32lakhs/cmm	2.52±0.24lakhs/cmm
preeclampsia	2.14± 0.39 lakhs/cmm.	2.33± 0.19 laks/cmm.	2.41±0.08lakhs/cmm
diabetes	2.27±0.48 lakhs/cmm	2.19±0.53 lakhs/cmm	2.37±0.16 lakhs/cmm

Out of 11 babies having thrombocytopenia, 5 babies had platelet counts less than1 lakh while the rest had counts between 1-1.5 lakhs. Only one baby had counts below 50,000 when compared to a mean of 2.57 lakhs/cmm in babies born to mothers without pregnancy induced hypertension.

Fetal hemoglobin

The mean fetal hemoglobin as estimated by the alkali denaturation method in all infants was 64.6%, In babies of 32-37 weeks gestation it was 67.6%, in neonates of <32 weeks gestation it was 85.3%.

DISCUSSION

Umbilical cord blood is supposed to be a rich source of mesenchymal stem cells, haematopoetic and non-haematopoetic stem cells.[6,7] Recent researches in stem cells have indicated that umbilical cord blood may be a significant source of laboratory sample for analysis of fetal haematologic parameters instead of newborn blood which is very difficult to obtain. The effect of maternal diseases like diabetes, anaemia and hypertension on the fetal haematology may also be evident in the umbilical cord blood.[8,9]

As there is a paucity of studies describing the normative values of haematological profile of neonates derived from umbilical cord blood in this part of the country, it was imperative that we make an attempt to develop the same to help paediatricians and neonatologists. The present study is the first of its kind with analysis of all the parameters in a tertiary care centre in Eastern India. Review of available literature yields some interesting facts. Firstly, the haematological values show a linear increase with the gestational age as expected.[10] The hemoglobin concentration of umbilical cord blood is certainly lower than European countries but higher than most African countries.[11-14] The same holds true for haematocrit. The mean corpuscular volume (MCV) is higher in our population than in Iranian and Italian studies.[15-17]

The haematologic parameters also exhibit statistically significant deviation in mothers affected by anaemia, hypertension and gestational diabetes. There is a wide variation in leucocyte count. The platelet count was performed in all cases. The mean and standard deviation of cord blood platelet count of all the babies was 2.51±0.34 laks /cmm. Oski et al had found a value of 2.9 laks/cmm as the mean platelet count in cord blood.[18] Sandhya Sivkumar et al had found a value of 2.09 laks/cmm.in case of term newborn infants.[19] The wide variation in the leucocyte count and the platelets could be accounted for by the following factors-environmental influences, effects of crushing during cutting the umbilical cord, mixing of the cord blood with Wharton's jelly and the different methods of estimation of blood counts and platelets (manual or automated).

In the present study nRBC count was performed in all cases in the stained slide prepared from the cord blood. nRBC counts were higher in pregnancy induced hypertensive mothers and in gestational diabetics. In 1944, Miller et al first reported the presence of increased

nRBCs and extramedullary erythropoiesis in infants of diabetic mothers. Infants of diabetic mothers who are large for gestational age have higher nRBC counts than those who are of appropriate size for gestational age.[20] The increased erythropoiesis is probably due to both an increase in erythropoietin levels and a direct hemopoietic effect of hyperinsulinaemia.[21-23]

The importance of estimation of alkali resistant hemoglobin cannot be over emphasized in a resource poor country like India which also has the dubious distinction of having a prevalence of thalassemia.[24,25] While majority of the hemoglobin in the fetus is alkali resistant, the proportion rapidly decreases in the first year of life. Literature search failed to yield any recent studies to measure alkali resistant hemoglobin estimates in India. In one study, the authors showed a significant increase of Hb F in 30-35 weeks preterm newborns.[26]

Last but not the least, the estimation of fetal WBC count also deserves a special mention. In a particular study, the total fetal leucocyte count increased exponentially from 2.8X109/l at 18 weeks to 11.8X109/l at term along with neutrophils, but lymphocyte and monocyte counts increased linearly.[27] There were no significant changes with gestation in early myeloid cells, eosinophils, and basophils .The physiological leucopenia observed in fetuses early in the third trimester may partly explain the predisposition of premature neonates to infection.

The values obtained in this study are quite different from those described in literature which may be explained by geographical variation, ethnicity, socioeconomic factors, etc. As this study was a part of a dissertation submitted for post graduate degree, the sample size was very small, and this is the main limitation of the study.

Large scale well designed studies with a much larger sample size need to be included to set up reference values of the haematological profile of the neonates born to mothers residing in this area. Further, variations in the haematological profile in the umbilical cord blood in neonates of mothers affected by anaemia, diabetes and hypertension would guide neonatologists attending these babies. The data generated by this study would help the personnel involved in storage of umbilical cord blood for the purpose of stem cell transplant. Simple analysis of these values of the haematological profile of the umbilical cord blood may eliminate the need of screening of the umbilical cord blood for preservation.

CONCLUSION

A small initiative was taken in a tertiary care hospital in eastern India to assess the haematological profile parameters in umbilical cord blood including alkali resistant hemoglobin in neonates at birth in relation to their gestational ages and maternal diseases. A cursory glance through the results would show that the values are significantly different from that available in literature. Normative values differ with respect to geographical areas, ethnicity, diet etc. and hence the need of the hour is to develop reference values of all the parameters of the haematological profile of neonates in this part of the country. This attempt may be considered as a stepping stone in this direction. In the parting note, the authors end here with the intention to carry out large scale, better designed studies with a larger sample size to achieve this objective, which will ultimately guide neonatologists, pediatricians and hematologists catering to this population.

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