HEMATOLOGICAL PROFILE IN NEONATES–A HOSPITAL BASED STUDY

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ABSTRACT: Neonatal hematology is a complex subspecialty of pediatric hematology, combining the unique aspects of maternal/fetal relationship, the delicate balance of coagulation factors and the distinctive physiologic condition of the newborn period. There are important hematological differences between the newborn, older child and adult. A careful assessment of the blood elements is often the first step in assessment of hematologic function and diagnosis of hematological disorders. Most of routine hematological parameters provide us with useful information that can help in diagnosing hematological disorders. The study was conducted in Department of Pathology and Department of Pediatrics, ASCOMS over a period of one year i.e from 1st November 2013 to 31st October 2014. All neonates, admitted in the pediatric ward during this period were included for the study. . For each patient following lab. tests were carried out which include hemoglobin estimation, total leucocyte count (TLC), differential leucocyte count(DLC), platelet count, hematocrit (PCV), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration(MCHC), reticulocyte count and whenever required coagulation tests (Prothrombin time and Activated partial thromboplastin time), sickling test (Metabisulfite slide test), glucose 6 phosphate dehydrogenase(G6PD) and C-reactive protein(CRP) were conducted. The aim of the study was to know the hematological profile of neonates, to ascertain the relative frequency of common hematological disorders and to draw clinico pathological correlations. Considering the variability of presentation and confounding factors, it is worthwhile to study hematological parameters in neonates to know the pattern of hematological diseases in this geographical area. This information can help us in better patient management.

KEYWORDS: Neonates, Hematology, Anemia, Haematological Disorders.

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INTRODUCTION: Neonatal hematology is a complex subspecialty of pediatric hematology, combining the unique aspects of maternal/fetal relationship, the delicate balance of coagulation factors and the distinctive physiologic condition of the newborn period. Neonatal hematology is a fast growing field and hematological problems occur in the majority of sick neonates.

There are important hematological differences between the newborn, older child and adult. These are related to embryological development of fetus, the interaction of the fetus and the mother and the changes necessary to adapt to extra uterine life (1) . Deranged hematological manifestations in the neonate can be classified into:

1. RBC disorders
2. WBC disorders and
3. Hemorrhagic disorders

A careful assessment of the blood elements is often the first step in assessment of hematologic function and diagnosis of hematological disorders.

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Blood elements include red cells, leucocytes and platelets.Evaluation of blood requires quantitation of each of cellular elements by either automated cell counter or manual method.

Most of routine hematological parameters provide us with useful information that can help in diagnosing hematological disorders.

AIMS AND OBJECTIVES:

1. To study the hematological profile of neonates admitted in Pediatric ward of Acharya Shri Chander College of Medical Sciences (ASCOMS)& Hospital, Jammu.
2. To find the relative frequency of hematological disorders in the neonates.
3. To correlate the clinical presentation with hematological disorders of neonates.

MATERIAL AND METHODS: The study was conducted in Department of Pathology and Department of Pediatrics, ASCOMS over a period of one year i.e. from 1st November 2013 to 31st October 2014. The entire study is prospective.

ELIGIBILITY CRITERIA: All neonates, admitted in the pediatric ward during this period were included for the study. The clinical records of all the patients fitting into eligibility criteria were obtained from the ward and an analysis of each patient's clinical profile with regard to age, gestation, sex was made.

The physical findings that were studied include apnea, anterior fontanelle, pallor, jaundice, bleeding manifestation like purpura, petechiae, gastrointestinal bleed, rectal bleed, umbilical stump bleed, blood stained froth from the mouth. For each patient following lab. Tests were carried out which include hemoglobin estimation (by Cyanmethemoglobin method)[2], total leucocyte count (TLC) (by Neubauer Chamber), differential leucocyte count(DLC)[2], platelet count (by Neubauer Chamber), hematocrit (PCV) (by Wintrobe's Methods), mean corpuscular volume(MCV), mean corpuscular hemoglobin(MCH), mean corpuscular hemoglobin concentration(MCHC).

Peripheral blood film (2) was stained with Leishman's stain and reticulocyte count were studied on films made with brilliant cresyl blue and whenever required coagulation tests (Prothrombin time and Activated partial thromboplastin time), sickling test (Metabisulfite slide test) (3), glucose 6 phosphate dehydrogenase(G6PD)(4) and C-reactive protein(CRP) were conducted. All biochemical, radiological, microbiological and serological investigations done were noted as mentioned in the performa.

Under all aseptic precautions, venous blood was collected for the study. EDTA was used as an anticoagulant for hematological profile and sodium citrate for coagulation tests. Hemoglobin estimation, total leucocyte count (TLC), differential leucocyte count (DLC), platelet count and hematocrit were done using automated cell counter and reconfirmed by their respective manual methods. Automated cell counter, Sysmex five part differential was used which works by the principle of impedance method.

The statistical analysis was done and the results are expressed as percentages and other appropriate statistical methods were applied wherever necessary.

Both cases of disseminated intravascular coagulation manifested clinically with gastrointestinal bleeding. Platelet counts were found reduced in both neonates. PT was abnormal in both neonates. APTT was deranged in 1 neonate.

DISCUSSION: The youngest patient was four hours old and the eldest 28 days old. Maximum number of patients 42(60%) was seen in the 0-72 hrs. age group. Of these 45 patients (64.29%) were male and 25 (35.71%) were female patients. The male: female ratio being 1.80:1. In 1988, Chandna et al studied the blood of 50 septicemic newborns [5]. In their study 58% of cases were males and the remaining were females. 26 (52%) neonates were less than 8 days old, 17 (34%) were in 8 -14 days age group, and only 7 (14%) neonates belonged to the 15-28 days age group. The male preponderance in these studies is because in the Indian setup male children are better looked after. It is a common problem in our society to encounter female infanticide and neglect of the female child.

In the present study out of 70 neonates 28 cases (40%) neonates had a normal hematological profile. The remaining 42 cases (60%) were found to have an abnormal hematological profile. The commonest hematological abnormality detected was anemia with 30 cases (42.86%) followed by septicemia in 13 cases (18.57%) and hemorrhagic disorders 7 cases (10%).

In the present study anemia was found to be the most common hematological abnormality with 30 cases (42.86%). Pallor was seen in 28 cases (93.33%). Jaundice was seen in 20 cases (66.67%) out of 30 cases of anemia. In this study 8 patients (26.67%) had splenomegaly and 2 patients (6.67%) had hepatomegaly. Evidence of bleed was present in 7 cases (23.33%).

The clinical manifestation of hemorrhage at birth depends upon the extent and duration of blood loss. When acute massive blood loss has occurred, the infant is extremely pale and requires immediate volume expansion and transfusion (Christensen, 2000)(6) . Apnea was seen in 6 cases (20%). Apneic spells frequently occur in premature infants. The incidence of apnea increases with decreasing gestational age.

Anemia of Infections is the commonest cause of anemia in the present series with 14. Both intrauterine and postnatally acquired infections are associated with anemia. In this study 9 cases had hemolytic diseases. This can be due to variety of factors intrinsic and extrinsic to the RBC. Post Hemorrhagic Anemia was seen in 4 cases. Faxelius et al (1972) reported that as many as one fourth of patients admitted to newborn intensive care units have a 20 to 30% decrease in their red blood cell volume because of hemorrhage [7]. In the series in 3 cases the cause of anemia could not be determined as these patients did not have features suggestive of hemorrhage, hemolysis or infection. Prevalence of this anemia(unclassified) is (4.29%).

In this series 4 cases were found to have hemorrhage as the cause of anemia. All cases of Hemolytic disease were jaundiced. The prevalence of G6PD deficiency in the present series was (5.71%) ABO incompatibility was (1.43%) and Rh incompatibility was (5.71%). Patil and Gupta (1995) reported an incidence 5.2 and 11.6 per 1000 live births for ABO Hemolytic disease of newborn in two hospital based studies from Bombay (8). Zipursky (1987) reported a prevalence of 8% for the Rh negative genotype in the Indian population (9).

The severity of hematologic abnormalities in neonates with Hemolytic anemia varies directly with the severity of hemolysis and the compensatory ability of the bone marrow. The hemoglobin may be normal despite a degree of hemolysis causing significant hyperbilirubinemia. The situation may reflect the adequately increased production of erythrocytes by the neonates to compensate for hemolysis (Phibbs et al, 1974) (10).

Anemia of infection is the commonest anemia. In this series it constitutes (46.67%) of all anemia. Zipursky & Jaber (1978) noted a fall in hemoglobin in patients suffering from severe bacterial infections and many of them had a sustained hyperbilirubinemia[11]. They suggested that Hemolytic anemia could arise by sensitization of the infants erythrocytes to substances produced by bacteria. In the present series the Hb of patients with anemia of infection was 11.9 ± 2.18 (gm/dl). As discussed by Buchanan et al (1985) the anemia of acute infection is generally mild [12]. Neonatal septicemia is one of the major factors contributing to the high perinatal or neonatal mortality and morbidity. Several authors have reported that the mortality due to neonatal sepsis ranges between 40 - 65% (Khatau et al, 1986) (13).

In a study by Chandna et al (1988) to evaluate various rapid diagnostic tests in neonatal septicemia 50 clinically suspected cases of septicemia and 10 healthy neonates were

studied. C-reactive protein (CRP) test, total leucocyte count, ratio of band cells: total polymorphonuclear cells, buffy coat smear examination for organisms and gastric aspirate cytology for polymorphonuclear cells were the rapid tests performed.

In their study leucopenia (WBC < 5000/mm^3) was considered to be one of the parameters indicative of septicemia. They found leucopenia in 4 cases (17.41 %) out of 23 culture proven septicemic cases and in 3 (11.5%) out of 26 culture negative cases. In contrast none of the controls showed leucopenia. In the present study we had 31 cases of clinically suspected septicemia. Less than 20% of these cases were culture proven for septicemia. Of these 31 cases of clinically suspected septicemia 13 cases had laboratory parameters suggestive of septicemia.

The remaining 17 neonates did not show hematological parameters suggestive of septicemia the probable reason for this could be that they had already received antibiotic therapy from outside. 3 cases (23.08%) had a leucocyte count <5000/cumm. The band: total polymorphonuclear cell ratio was > 0.2 in 10 (76.92%) out of 13 cases in our study. In the study by Chandna et. al. (1988) the band to total polymorphonuclear cell ratio was >0.2 in 16 out of 23 (69.6%) cases of proven septicemia and 18 out of 26 cases (69.2%) of culture negative septicemia.

In the present study 7 neonates had hemorrhagic disorders. The diagnosis of hemorrhage in the newborn infant can at times be difficult. Infants can present with a spectrum of clinical characteristics, depending upon the degree of hypovolemia and anemia and on the timing of blood loss. Infants with acute hemorrhage are pale and tachycardic. The most common cause of impaired hemostasis and clinically apparent hemorrhage in a neonate is consumptive coagulopathy also known as disseminated intravascular coagulation or DIC. Neonates are pre-disposed to DIC because of immaturity of their mechanisms defense against excessive thrombosis. (Hathaway et al, 1975) [14].

SUMMARY AND CONCLUSION: The aim of the study was to know the hematological profile of neonates, to ascertain the relative frequency of common hematological disorders and to draw clinico pathological correlations. Considering the variability of presentation and confounding factors, it is worthwhile to study hematological parameters in neonates to know the pattern of hematological diseases in this geographical area. This information can help us in better patient management. Most of routine hematological parameters provide us with useful information that can help in diagnosing hematological disorders.

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OBSERVATIONS:

Age and Sex Distribution:

The age and sex of these neonates are as shown in Table-1

Sl. No.	Age	Sex				
		Males		Females		Total
		No. of cases	%	No. of cases	%	No. of cases
1.	0-72 Hrs.	26	37.14%	16	22.86%	42
2.	72 hrs. - 7 days	9	12.87%	3	4.28%	12
3.	8 - 14 days	4	5.71%	2	2.86%	6
4.	15 - 21 days	3	4.28%	2	2.86%	5
5.	22 - 28 days	3	4.28%	2	2.86%	5
	Total	**45**	**64.28%**	**25**	**35.72%**	**70**

Table I : Age & Sex Distribution

The youngest patient was 4 hours old and the eldest 28 days old. Maximum number of patients 42(60%) was seen in the 0-72 hrs. age group. Out of a total 70 patients 45(64.28%) were male patients and 25 (35.72%) were female patients. The male: female ratio being 1.80: 1.Out of 70 patients 28 (40%) neonates had a normal hematological profile. Table – II (Figure-II) lists the hematological parameters of these neonates.

Age	Hb (in gm/dl)	TLC (/cumm)	Platelets (lakh/cumm)	Retic Count (%)
0 to 72 hrs.	(18.96 ± 2.36)	(18092.86 ± 2491.16)	3.86 ± 0.46	4.21 ± 1.76
72 hrs. to 7 days	(16.66± 2.16)	(17840 ± 1792.2)	3.48 ± 1.08	3.5 ± 1.42
8 to 14 days	(15.53 ± 4.48)	(17666.67 ± 3055.04)	2.9 ± 1.96	3.47 ± 1.52
15 to 21 days	(14.65 ± 2.24)	(12875 ± 2650.14)	2.87 ± 1.56	3.32 ± 1.06
22 to 28 days	(14.5 ± 1.4)	(13250 ±3535.54)	2.75 ± 1.54	2.95 ± 2.12

Table II: Normal Hematological Parameters In The Neonatal Period

The remaining 42(60%) neonates were found to have an abnormal hematological profile. The various disease categories of neonates with abnormal hematological profile are listed in Table –III (Figure-1II).

Sl. No.	Disease Category	No. of cases observed	Percentage in the population under study
1.	RBC Disorders–Anemia	30	42.86%
2.	WBC Disorders–epticemia	13	18.57%
3.	Hemorrhagic disorders	7	10%

Table III: Disease Categories of Neonates with Abnormal Hematological Profile

Some neonates belong to more than one diseases category.

RBC Disorders: 1. Anemia: In the present series anemia was found to be the most common hematological abnormality 30 (42.86%) Anemia is defined as a Hb <13.7gm/dl (Christensen, 2000).

Table 1-A (Figure 1-A) lists the correlation of the various clinical features with anemia

Sl. No.	Clinical Features	No. of Cases	Percentage (of all anemia, Total = 30 case)
1	Pallor	28	93.33%
2.	Jaundice	20	66.67%
3.	Splenomegaly	8	26.67%
4.	Hepatomegaly (Liver > 2.0 cm below right costal margin)	2	6.67%
5.	Evidence of bleed, (Intracranial hemorrhage, GI bleed, Cephalhaematoma)	7	23.33%
6.	Apnea	6	20%

Table 1-A: Correlation of Clinical Features With Anemia

The causes of the various anemia and their respective prevalence in the present series is listed in Table 1-B (Figure 1-B).

Sl. No.	Type of Anemia	No. of Cases	Percentage of all anemia (Total 30 cases)	Percentage in this series (Total 70 cases)
I.	Post hemorrhagic Anemia	4	13.33%	5.71%
II.	Hemolytic Diseases	9	30%	12.86%
III.	Anemia of infection	14	46.67%	20%
IV.	Unclassified	3	10%	4.29%

Table 1-B: Causes of Anemia

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Post Hemorrhagic Anemia: 4 cases were found to have hemorrhage as the cause of anemia. Post hemorrhagic anemia constitutes 13.33% of all anemia. The percentage of post hemorrhagic anemia in this series is 5.71%.

Table 1-C (Figure 1-C) lists the causes of post-hemorrhagic anemia as seen in the series.

Sl. No.	Site of hemorrhage	No. of cases	Percentage of all post hemorrhagic anemia
1	Intracranial hemorrhage	2	50%
2	Gastro intestinal bleed	1	25%
3	Umbilical Cord bleed	1	25%

Table 1-C: Causes of Post-Hemorrhagic Anemia

Table 1-D lists the hematological parameters in post hemorrhagic anemia.

Laboratory Parameter	Value
Hb (in gm/dl)	10.87 ± 2.6
TLC (/cumm)	24700 ± 12213.64
Platelets (lakh/cumm)	3.92 ± 2.4
Retic count (%)	6.02 ± 1.1

Table 1-D: Hematological Parameters in Post Hemorrhagic Anemia

Hemolytic Anemia: 9 cases of Hemolytic anemia were diagnosed in the present series. The causes of hemolysis in present series are listed in Table 1-E (Figure 1-E).

Sl. No.	Cause of Hemolysis	No. of cases	Percentage of all hemolytic anemia (9 cases)	Percentage of Hemolytic anemia in this series (70 cases)
1	G6PD deficiency	4	44.44%	5.71%
2	ABO Incompatibility	1	11.12%	1.43%
3	Rh Incompatibility	4	44.44%	5.71%
	Total	**9**	**100%**	

Table 1-E: Causes of Hemolysis in The Neonatal Period

Table 1-F (Figure 1-F) list correlation of the clinical features and in the various hemolytic states.

Sl. No.	Clinical parameter under study	G6PD deficiency (Total 4 cases)		ABO incompatibility (Total 1 case)		Rh incompatibility (Total 4 cases)	
1	Jaundice	4	100%	1	100%	4	100.00%
2	Pallor	2	50%	1	100%	4	100.00%
3	Splenomegaly	2	50%	0		3	75%
4	Hepatomegaly	1	25%	0		1	25%

TABLE 1-F: Correlation Between The Clinical Features In The Various Hemolytic States

Jaundice was seen in all cases of Hemolytic anemia. Pallor was present in all cases in Rh incompatibility, 100% cases of ABO incompatibility and 50% cases of G6PD deficiency. Splenomegaly was present in 75% cases of Rh incompatibility, 50% cases of G6PD deficiency. Hepatomegaly was present in 25% of G6PD deficiency cases and 25% of cases of Rh incompatibility.

Table 1-G lists the hematological parameters in various hemolytic anemia.

Sl. No.	Laboratory parameter under study	G6PD deficiency (Total 4 cases)	ABO incompatibility (Total 1 case)	Rh incompatibility (Total 4 cases)
1	Hb (gm/dl)	12.15 ± 0.70	12.8	9.32 ± 2.1
2	TLC (/cumm)	22450 ± 11713.8	17300	13750 ± 4550.46
3	Platelets (in lakhs/cumm)	3.37 ± 2.18	4.2	1.92 ± 1.06
4	Retic count(%)	7.72 ± 1.62	10	10.37 ± 3.92
Table 1-G: Correlation Between The Laboratory Parameters in the Various Hemolytic States				

Anemia of Infection: Anemia of infection was the commonest anemia in this series and constituted 46.67% of all anemia. The prevalence of this anemia in this series is 20%.

Table 1-H (Figure 1-H) lists the clinical presentation in anemia of infection.

Sl. No.	Clinical Presentation	No. of cases	Percentage of all anemia of infection
1	Septicemia/probable septicemia	11	78.57%
2	Bronchopneumonia	1	7.14%
3	Septic arthritis	1	7.14%
4	Meningitis	1	7.14%
Table 1-H: Clinical Presentation in Anemia of Infection			

Anemia of infection is most often seen with clinically diagnosed septicemia 11 cases (78.57%) followed by bronchopneumonia, septic arthritis and meningitis 1 case (7.14%) each.

Table 1-I lists the hematological parameters in anemia of Infection.

Laboratory Parameter	Value
Hb (gm/dl)	11.9 ± 2.18
TLC (/cumm)	23557.14 ± 17774.44
Platelets (lakhs/cumm)	4.65 ± 3.3
Table 1-I: Hematological Parameters In Anemia Of Infection	

Unclassified: In 3 cases the precise cause of anemia could not be detected (10%).

Table 1-J lists the hematological parameters in unclassified anemia.

Laboratory Parameter	Value
Hb (gm/dl)	11.5 ± 2.88
TLC (/cumm)	21866.66 ± 9002.96
Platelets (lakh/cumm)	1.9 ± 1
Retic count (%)	3.73 ± 2.1
TABLE 1-J: Hematological Parameters In Unclassified Anemia	

WBC DISORDERS: 1. Neonatal Sepsis Septicemia is the next most common hematological abnormality.In this series out of 31 cases of clinically suspected septicemia 13 cases i.e 41.93% had hematological parameters suggestive of septicemia. The remaining 58.07% of patients with clinically suspected septicemia did not have hematological parameters suggestive of sepsis.

In our present study we used the two test combination recommended by Philip & Hewitt (1980). This states that leucopenia < 5000/cumm and band: total neutrophil ratio > 0.2, is strongly suggestive of septicemia.

Table 2-A list out the WBC findings suggestive of septicemia in the present series.

Sl. No.	Lab. Parameters	No. of cases	Percentage in 13 cases of septicemia
1.	Leucopenia(TLC < 5,000/cumm)	3	23.08%
2.	Band cell: Total neutrophil ratio > 0.2	10	76.92%
Table 2-A: Laboratory Parameters Suggestive Of Septicemia			

Table 2-B (Figure 2-B) lists the clinical features seen in patients with septicemia.

Sl. No.	Clinical Parameter	No. of cases	Percentage of all (13)cases of septicemia
1.	Pallor	7	53.85%
2.	Jaundice	6	46.15%
3.	Apnea	3	23.08%
4.	Splenomegaly	4	30.77%
5.	Hepatomegaly (liver>2.0cm below right costal margin	2	15.38%
6.	Evidence of bleed	2	15.38%
7.	Anterior fontanelle bulging	1	7.69%

Table 2-B: Clinical Features in Septicemia

Hemorrhagic Disorders: This is the third common hematological abnormality seen in the present series. 7 cases (10%) had hemorrhagic diseases.

Table 3-A (Figure 3-A) lists the site of hemorrhage in these cases.

Sl. No.	Site of Hemorrhage	No. of Cases	% of all cases (7) with hemorrhagic disorder
1.	Intracranial hemorrhage	4	57.14%
2.	G I bleed	2	28.57%
3.	Umbilical cord bleed	1	14.28%

Table 3–A: Site of Hemorrhage

Table 3-B (Figure 3-B) lists clinical features of these 7 patients.

Sl No.	Clinical features	No. of Cases	% of all (7) patients with hemorrhagic disorder
1.	Jaundice	4	57.14%
2.	Pallor	4	57.14%
3.	Anterior fontanelle fullness	2	28.57%
4.	Apnea	1	14.28%
5.	Splenomegaly	2	28.57%
6.	Hepatomegaly	1	14.28%

Table 3-B: Clinical features of Neonates with Hemorrhagic Disorders

Disseminated Intravascular Coagulation:Two neonates were found to have disseminated intravascular coagulation .The prevalence of DIC in this series was 2.86%.

Table 3-C lists the clinical features and laboratory parameters of neonates with disseminated intravascular coagulation.

Sl. No.	Clinical Presentation	Platelet Count	Total Leucocyte count	PT	APTT
1	GI Bleed	90,000/ cumm	9900	T-23 C-14	T-52 C-37
2	GI Bleed	40,000/ cumm	6800	T-20 C-13	T-35 C-33

Table 3-C: Clinical Presentation and Laboratory Parameters of Disseminated Intravascular Coagulation