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Research Article

TOTAL AND DIFFERENTIAL LEUKOCYTE COUNT IN HEALTHY NEWBORNS

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ABSTRACT

Objectives: The objectives of the study were to establish normal reference values of total and differential leukocyte count (DLC) in Indian neonatal population.

Methods: The study was conducted on 200 healthy newborns admitted to neonatology section of Department of Paediatricsa. Maternal and Neonatal data was recorded on a pretested pro forma. Thin-layer chromatography (TLC) was done by manual method (hemocytometer method) using improved Neubauer Chamber. For calculating DLC smear was made from the EDTA blood and was stained by Leishman stain (Romanowsky group). DLC was done in body and tail part of the stained smears by following Z pattern.

Statistical Analysis: Descriptive statistical analysis was carried out in the present study. Mean, standard deviation, t-test, and analysis of variance tests were used to test the significance of the differences among sample means.

Results: Mean TLC at various period of gestations were 4500/cumm at 31 weeks, 7627±2320/cumm at 33 weeks, 8325±1943/cumm at 34 weeks, 8141±1538/cumm at 35 weeks, 8455±904/cumm at 36 weeks, 8840±658/cumm at37 weeks, 9380±896/cumm at 38 weeks, 10143±1369/cumm at 39 weeks, 9155±2444/cumm at 40 weeks, and 11750±353/cumm at 41 weeks.

Conclusion: TLC showed increase with gestation except at 35 weeks and 40 weeks. Neutrophils shows increase with gestation except at 35 weeks and 40 weeks lymphocytes shows increase with gestation except at 34 weeks, 39 weeks, and 40 weeks.

Keywords: Thin-layer chromatography, Differential leukocyte count, Gestation, Newborns.

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INTRODUCTION

It is common knowledge that a person's health varies with time, age, and location. This level is thus relative and not absolute. This implies that individuals' conditions must be correlated with or compared to reference data. Individuals' conditions may be evaluated by comparing information obtained through medical interviews, clinical examinations, and supplementary investigations with reference data [1].

In recent decades, the hematological profile of neonates has remained a topic of intense research. The increased acknowledgment of the significance of gestation and intrauterine fetal growth retardation with probable differences in these several groups of newborns has led to further research in this field [2]. The white blood cell (WBC) count and platelet count have been useful in evaluating neonatal sepsis and newborn coagulation status [3].

Numerous studies have been performed, including one author examining the peripheral leukocyte count and leukocyte indices in term-born healthy newborns [4]. This research aimed to establish normal values for the peripheral leukocyte count and leukocyte index in term newborns at a certain period after delivery. Another study investigated all hematological parameters, such as Hb, Hematocrit levels, WBC, Platelet, and RBC counts, in spontaneously delivered newborns [5]. It was determined that higher parity and pregnancy were related to reducing all hematological parameters in cord blood, except the lymphocyte count.

In addition, another research on neonatal cellular analysis revealed that the total WBC and differential counts of term newborns are considerably different from those of older children and adults [6]. It was discovered that term infants are born with a high WBC count and relative neutrophilia.

Except for changes in the number of leucocytes (WBC) in response to infection, white cell diseases are uncommon in newborns. Normal levels for neutrophils at birth are also altered by prenatal and perinatal history and ethnic background. Transient neutropenia is prevalent in premature newborns but rare in full-term infants. Neutrophil numbers fluctuate with both pregnant and postnatal age, especially during the 1st few days of life [7].

In infants, hematological parameters are commonly measured for diagnostic reasons when infections and bleeding abnormalities are suspected. The WBC count has shown useful in the diagnosis of newborn sepsis. Consequently, determining the reference range for total and differential WBC counts is clinically significant [8].

Consequently, this research aims to establish total and differential WBC count reference values for healthy neonates utilizing umbilical cord blood.

METHODS

The research was conducted on 200 healthy babies admitted to the neonatology unit of a tertiary hospital's department of pediatrics.

Informed consent

After obtaining the informed written consent of the parents of the infants, data on the mother and the baby were entered into a pretested pro forma.

Exclusion criteria

The following situations were excluded from the study: Pregnancy induced hypertension, gestational diabetes, fever, prolonged labor, preterm rupture of membranes, foul-smelling liquor, dai handling, multiple vaginal examinations, and instrumentation during delivery. Septicemia, birth asphyxia, respiratory distress syndrome, blood group incompatibility, intraventricular hemorrhage, congenitally deformed newborns, small for gestational age, and large for gestational age were all excluded from the research, as were babies born with any of these problems. From the 1st day of the last menstrual cycle until the day of the baby's birth, gestational age was determined. The New Ballard Scoring System (NBSS) was used as a benchmark in the comparison. It was regarded as usual to have a discrepancy ±2 weeks.

EDTA-containing vials were used to collect a 2 ml cord blood sample after delivery. Total leukocyte count and differential leukocyte count (DLC) were both performed using EDTA blood. An updated Neubauer Chamber was used to perform Thin-layer chromatography (TLC), done manually using the hemocytometer technique. A WBC pipette was used to draw blood up to the 0.5 mark. Up to and including the 11th drop of the WBC dilution liquid (Turk's fluid). The WBC pipette was loaded into Neubauer's chamber. Four big corner squares were counted with a modest power goal (20×) for WBCs. A calculation was made to determine the average amount of WBC in a given blood volume. Bloodstained with Leishman stain was used to make a smear in the DLC calculation (Romanowsky group). DLC was applied to the stained smears in the body and tail using the Z pattern[9].

RESULTS

The study was conducted in the neonatology section of the department of paediatrics, in association with the department of clinical pathology, of tertiary care hospital. In this study, 200 healthy newborns were taken as subjects. TLC and DLC measurements were performed manually (using a hemocytometer) using an upgraded Neubauer Chamber on a sample of cord blood. Following the Z pattern, DLC was performed on the body and tail of a smear generated from EDTA blood stained with Leishman stain (Romanowsky group).

Mean TLC at various periods of gestations was 4500/cumm at 31 weeks, 7627±2320/cumm at 33 weeks, 8325±1943/cumm at 34 weeks, 8141±1538/cumm at 35 weeks, 8455±904/cumm at 36 weeks, 8840±658/cumm at37 weeks, 9380±896/cumm at 38 weeks, 10143±1369/cumm at 39 weeks, 9155±2444/cumm at 40 weeks, and 11750±353/cumm at 41 weeks. There was no baby born with 32 weeks POG. Statistical difference between mean TLC in babies at various periods of gestations was highly significant. Mean DLC at various periods of gestations was calculated. Neutrophils (/cumm) were 2600, 3692 ±2166, 4751±2267, 4159±668, 4059±874, 4449±1072, 4647±786, 5834±1389, 5750±2530, and 6865±3528 at 31 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks,37 weeks, 38 weeks, 39 weeks, 40 weeks, and 41 weeks, respectively. The statistical difference at various periods of gestations was highly significant. Lymphocytes (/cumm) were 1890, 3841±989, 3396±613, 3693±1232, 4049±937, 4226±811, 4503±882, 4267±1005, 3321±1563, and 4885±3174 at 31 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks, 37 weeks, 38 weeks, 39 weeks, 40 weeks, and 41 weeks, respectively. The statistical difference at various periods of gestations was significant. Monocytes (/cumm) were 0, 93±144, 120±127, 102±128, 140±163, 73±151, 139±192, 35±69, and 92±172 and 0 at 31 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks, 37 weeks, 38 weeks, 39 weeks, 40 weeks, and 41 weeks, respectively. The statistical difference at various periods of gestations was not significant. Eosinophils (/cumm) were 0, 67±106, 34±83, 64±100, 100±126, 34±68, 86±117, 23±574, and 66±100 and 0 at 31 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks,37 weeks, 38 weeks, 39 weeks, 40 weeks, and 41 weeks, respectively. The statistical difference at various periods of gestations was significant. Basophils (/cumm) 0, 9±25, 22±55, 47±103, 46±81, 7±24, 30±54, 2±15, and 34±51 and 0 at 31 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks,37 weeks, 38 weeks, 39 weeks, 40 weeks, and 41 weeks, respectively. The statistical difference at various periods of gestations was significant. Immature cells (/cumm) were 0, 0, 0, 0, 8±25, 0, 3±18, 0, and 11±33 and 0 at 31 weeks, 33 weeks, 34 weeks, 35 weeks, 36 weeks,37 weeks, 38 weeks, 39 weeks, 40 weeks, and 41 weeks, respectively. The statistical difference at various periods of gestations was not significant (Table 1).

When babies were grouped according to the maturity, that is, mean TLC in babies born at the period of gestations <37 weeks was 8140±1571/cumm and at 37–42 weeks was 9515±1279/cumm. The statistical difference between the two groups was highly

Table 1: TLC and DLC with periods of gestation n=200

Period of Gestation	f	TLC	DLC					
		Mean SD (Reference range)	Mean SD (Reference range)					
			N	L	M	E	B	I
31	1	4500 (4500)	2600 (2600)	1890 (1890)	0 (0)	0 (0)	0 (0)	0 (0)
33	8	7627±2320 (6000–13100)	3692±2166 (1643–8515)	3841±989 (2190–5236)	93±144 (0–365)	67±106 (0–292)	9±25 (0–73)	0 (0)
34	6	8325±1943 (6800–11800)	4751±2267 (2720–8968)	3396±613 (2714–4375)	120±127 (0–340)	34±83 (0–204)	22±55 (0–136)	0 (0)
35	18	8141±1538 (7200–13400)	4159±668 (2960–5476)	3693±1232 (1924–7504)	102±128 (0–440)	64±100 (0–352)	47±103 (0–370)	0 (0)
36	21	8455±904 (6400–10900)	4059±874 (2432–5920)	4049±937 (2080–6104)	140±163 (0–510)	100±126 (0–436)	46±81 (0–320)	8±25 (0–85)
37	35	8840±658 (7200–11600)	4449±1072 (3168–7600)	4226±811 (1936–5162)	73±151 (0–720)	34±68 (0–261)	7±24 (0–90)	0 (0)
38	53	9380±896 (6800–12400)	4647±786 (1032–6448)	4503±882 (2072–7568)	139±192 (0–960)	86±117 (0–510)	30±54 (0–192)	3±18 (0–98)
39	47	10143±1369 (5800–12800)	5834±1389 (2730–9150)	4267±1005 (2668–7776)	35±69 (0–236)	23±57 (0–244)	21±15 (0–104)	0 (0)
40	9	9155±2444 (4500–12000)	5750±2530 (2520–9600)	3321±1563 (1600–6400)	92±172 (0–500)	66±100 (0–300)	34±51 (0–120)	11±33 (0–100)
41	2	11750±353 (11500–12000)	6865±3528 (4370–9360)	4885±3174 (2640–7130)	0 (0)	0 (0)	0 (0)	0 (0)
ANOVA F value		9.639	7.338	3.089	1.810	2.103	2.240	1.129
p-value		<0.001	<0.001	0.002	0.069	0.031	0.021	0.344

NB: NS=Non-significant, *=Significant at 0.01 level, TLC: Thin-layer chromatography, DLC: Differential leukocyte count

significant. Mean TLC in babies at birth was 9143±1491. Mean DLC in babies born at gestations <37 weeks and 37–42 weeks was calculated. Neutrophils were 4088±1286/cumm at <37 weeks, were 5080±1401/cumm at 37–42 weeks, and in all newborns at birth were 4812±1437. The statistical difference between the two groups was highly significant. Lymphocytes were 3787±1048/cumm at <37 weeks, were 4293±1019 at 37–42 weeks, and in all newborns at birth were 4156±1049. The statistical difference between two groups was significant. Monocytes were 115±142/cumm at <37 weeks, was 85±154/cumm at 37–42 weeks and in all newborns at birth was 93±151. The statistical difference between two groups was not significant. Eosinophils were 74±109/cummat<37 weeks, were 51±92/cumm at 37–42 weeks, and in all newborns at birth were 57±97. The statistical difference between two groups was not significant. Basophils were 37±81 at <37 weeks, were 15±40 at 37–42 weeks, and in all newborns at birth were 21±54. The statistical difference between two groups was significant. Immature cells were 3±16/cumm at <37 weeks, were 1±13/cumm at 37–42 weeks, and in all newborns at birth were 2±14. The statistical difference between two groups was not significant (Table 2).

The difference between male and female Mean TLC/DLC was statistically not significant except for monocytes (Table 3).

Mean TLC at different gestation periods according to NBSS was also calculated. It was 8300±4386/cumm at 32–34 weeks, 8369±1781/cumm at 34–36 weeks, 9035±1163/cumm at 36–38 weeks, and 4765±1456/cumm at 38–40 weeks. Statistical difference in mean TLC at different gestation periods according to NBSS was highly significant. However, the statistical difference for DLC in four groups was significant only for neutrophils (Table 4).

DISCUSSION

The current research comprised 200 healthy neonates admitted to the neonatology division of a tertiary hospital's department of pediatrics. A sample of cord blood was obtained at birth, and TLC and DLC were measured. Several researchers investigated the significance of TLC and DLC in babies [10-12].

Besides being of academic interest, the normal values at birth should be standardized so that infants at risk can be screened. There was

Table 2: TLC and DLC relation to gestation (weeks) n=200

Period of Gestation	f	TLC	DLC					
		Mean SD (Reference range)	Mean SD (Reference range)					
			N	L	M	E	B	I
<37 weeks	54	8140±1571 (4500–13400)	4088±1286 (1643–8968)	3787±1048 (1890–7504)	115±142 (0–510)	74±109 (0–436)	37±81 (0–370)	3±16 (0–85)
37–42 weeks	146	9515±1279 (6000–12800)	5080±1401 (1032–9600)	4293±1019 (1600–7776)	85±154 (0–960)	51±92 (0–510)	15±40 (0–192)	1±13 (0–100)
ANOVA F value		40.07	20.62	9.55	1.587	2.231	6.572	0.240
p-value		<0.001*	<0.001*	0.002*	0.209^{NS}	0.137^{NS}	0.011^{NS}	0.624^{NS}

NB: NS=Non-significant, *=Significant at 0.01 level, TLC: Thin-layer chromatography, DLC: Differential leukocyte count

Table 3: TLC and DLC with gender n=200

Gender	f	TLC	DLC					
		Mean SD (Reference range)	Mean SD (Reference range)					
			N	L	M	E	B	I
Male	101	9081±1599 (4500–13100)	4817±1511 (1032–9600)	4104±1106 (1800–7568)	68±117 (0–612)	48±98 (0–510)	14±49 (0–370)	1±11 (0–85)
Female	99	9207±1377 (5800–13400)	4807±1365 (1643–9360)	4209±990 (1600–7776)	119±177 (0–960)	66±96 (0–392)	28±59 (0–320)	2±16 (0–100)
ANOVA F value		0.353	0.003	0.500	5.828	1.660	3.013	0.386
p-value		0.553^{NS}	0.958^{NS}	0.480^{NS}	0.017^{NS}	0.199^{NS}	0.084^{NS}	0.535^{NS}

NB: NS=Non-significant, *=Significant at 0.01 level, TLC: Thin-layer chromatography, DLC: Differential leukocyte count

Table 4: TLC and DLC in relation to NBSS (POG) n=200

Weeks	f	TLC	DLC					
		Mean SD (Reference range)	Mean SD (Reference range)					
			N	L	M	E	B	I
32–34	3	8300±4386 (4500–13100)	5165±3034 (2600–8515)	2888±1476 (1890–4585)	121±210 (0–365)	97±168 (0–292)	24±42 (0–73)	0 (0)
34–36	22	8369±1781 (6320–13400)	4277±1471 (1643–8968)	3879±1214 (1924–7504)	108±102 (0–268)	63±81 (0–212)	10±27 (0–86)	0 (0)
36–38	97	9035±1163 (5800–12400)	4644±1094 (2432–7192)	4218±755 (1936–5996)	97±175 (0–960)	56±104 (0–510)	26±68 (0–370)	1±12 (0–92)
38–40	62	9765±1456 (4500–12800)	5408±1705 (1032–9600)	4213±1327 (1600–7776)	85±132 (0–564)	49±76 (0–300)	18±40 (0–176)	3±17 (0–100)
ANOVA F Value		6.585	5.201	2.150	0.159	0.337	0.619	0.334
p value		<0.001*	0.002*	0.096^{NS}	0.924^{NS}	0.799^{NS}	0.604^{NS}	0.801^{NS}

NB: NS=Non-significant, *=Significant at 0.01 level

much confusion in the early part of the century about normal value because of differences in estimation methods and modes of expression. With the advent of better techniques in hematology and uniformity in expression, this has largely been solved.

In the present study, the values of mean TLC and DLC at different gestation periods were at 31 weeks, TLC was 4500/cumm, and DLC was N-2600/cumm, L-1890/cumm, M-0/cumm, E-0/cumm, B-0/cumm, and I-0/cumm. At 33 weeks, TLC was 7627±2320/cumm and DLC was N-3692±2166/cumm, L-3841±989/cumm, M-93±144/cumm, E-67±106/cumm, B-9±25/cumm, and I-0/cumm. At 34 weeks, TLC was 8325±1943/cumm and DLC was N-4751±2267/cumm, L-3396±613/cumm, M-120±127/cumm, E-34±83/cumm, B-22±55/cumm, and I-0/cumm. At 35 weeks, TLC was 8141±1538/cumm and DLC was N-4159±668/cumm, L-3693±1232/cumm, M-102±128/cumm, E-64±100/cumm, B-47±103/cumm, and I-0/cumm. At 36 weeks, TLC was 8455±904/cumm and DLC was N-4059±874/cumm,L-4049±937/cumm, M-140±163/cumm, E-100±126/cumm, B-46±81/cumm, and I-8±25/cumm. At 37 weeks, TLC was 8840±658/cumm and DLC was N-4449±1072/cumm, L-4226±811/cumm, M-73±151/cumm, E-34±68/cumm, B-7±24/cumm, and I-0/cumm. At 38 weeks, TLC was 9380±896/cumm and DLC was N-4647±786/cumm, L-4503±882/cumm, M-139±192/cumm, E-86±113/cumm, B-30±54/cumm, and I-3±18/cumm. At 39 weeks, TLC was 10143±1369/cumm and DLC was N-5834±1389/cumm, L-4267±1005/cumm, M-35±69/cumm, E-23±57/cumm, B-21±15/cumm, and I-0/cumm. At 40 weeks, TLC was 9155±2444/cumm and DLC was N-5750±2530/cumm, L-3321±1563/cumm, M-92±172/cumm, E-66±100/cumm, B-34±51/cumm, and I-11±33/cum. At 41 weeks, TLC was 11750±353/cumm and DLC was N-6865±3528/cumm, L-4885±3174/cumm, M-0/cumm, E-0/cumm, B-0/cumm, and I-0/cumm. TLC showed an increase with gestation except at 35 weeks and 40 weeks. Neutrophils show an increase with gestation except at 35 weeks and 40 weeks. Lymphocytes show an increase with gestation except at 34 weeks, 39 weeks, and 40 weeks. The present study results were comparable to those of Davies *et al*. [13]. The observed changes in the number of circulating leucocytes and changes in other fetal hematological values with gestation presumably reflect alterations in the differentiation pattern of pluripotent hemopoietic stem cells to meet changing fetal physiological priorities during gestation [14,15]. Few studies observed the value of neutrophils as 3600/cumm and lymphocytes as 4200/cumm in babies born at 29–40 weeks of gestation [10,11]. The findings were comparable to our study.

Multiple authors studied the mean TLC and DLC value in term and preterm [16-18,8]. Abdurrahman *et al*. studied the value of the TLC in full-term newborns as 9520±6450/cumm. Xanthou *et al*. found the value of TLC in full-term newborns as 9700/cumm and DLC as N-4100/cumm and L-3900/cumm, and Rosse *et al*. found the value of TLC in full-term newborns as 11500/cumm and DLC as N-6500/cumm and L-3500/cumm. The value of mean TLC in present study in preterm newborns was 8140±1571/cumm and in term newborns was 9515±1279/cumm and these results were comparable to the above studies. On the other hand, values of mean DLC in the present study were comparable to Xanthou *et al*. and Rosse *et al*.

In the present study, the mean TLC in males and females was 9081±1599 and 9207±1377/cumm, respectively. The difference in mean TLC between male and female groups was statistically not significant. The statistical difference for DLC in the two groups was significant only for monocytes. No author had studied the correlation of sex with TLC and DLC.

In the present study, mean TLC at different periods of gestation according to NBSS was 8300±4386, 8369±1781, 9035±1163, and 4765±1456/cumm at 32–34 weeks, 34–36 weeks, 36–38 weeks, and 38–40 weeks, respectively. Statistical difference in mean TLC between different gestation periods according to NBSS was significant. The statistical difference for DLC in four groups was significant only for neutrophils. No author had studied the correlation of gestation periods according to NBSS with TLC and DLC in newborns.

CONCLUSION

TLC showed increase with gestation except at 35 wks and 40 wks. Neutrophils shows increase with gestation except at 35 wks and 40 wks Lymphocytes shows increase with gestation except at 34 wks, 39 wks and 40 wks.

AUTHORS CONTRIBUTION

Dr Ashwani kumar conceptualized and carried out the study. In addition to this, Dr Meetu yadav, Dr Gopalsinghcharanand Dr Pankaj sarangalparticipated in data collection, review literature, and data analysis.

DECLARATION OF CONFLICTING INTERESTS

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