RESEARCH PAPER

Immunoglobulin Profile and Lymphocyte Subsets in Preterm Neonates

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Objective: We documented the immunological profile of neonates and mothers, and lymphocyte subsets at birth. **Methods**: Consecutively born preterm neonates (26 to 31 weeks gestation) at our level III neonatal unit, fulfilling the inclusion criteria were enrolled. Immunoglobulin levels were assessed in maternal blood and in cord blood along with T cell subsets. **Results**: A total of 115 neonates were enrolled. The mean cord levels for IgG, IgM and IgA, respectively were 5.34, 0.10 and 0.04 g/L and of B, T, NK and NK-T cells were 14%, 71%, 10% and 1%, respectively of total lymphocyte population. Cord IgG and IgA levels showed a significantly rising trend with increasing gestation (*P*=0.005 and 0.02, respectively) but not IgM and T cell subsets. Maternal immunoglobulins were similar in all gestations. **Conclusion**: The cord IgG and IgA increased with increasing gestation but not IgM in neonates.

Keywords: *B cells, Gestation, NK-T cells, T cells.*

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Preterm neonates, born before 37 completed weeks of gestation, constitute 11% of total births with those born before 32 weeks comprising about 16% of preterm births [1,2]. Preterm neonates are seen to develop sepsis more often; one of the factors being immature immune system. Previous studies on immuno-globulin profile in preterm neonates have documented reduced immunoglobulin concentrations in preterm as compared to term babies. Studies on lymphocyte subsets and immunoglobulins in preterm neonates have had varying results [3-15]. Literature is scanty regarding immuno-globulin and lymphocyte subsets at very low gestation. Hence, our aim was to study the immunoglobulin profiles and lympho-cyte subsets in very preterm neonates at birth, which may have clinical implications.

METHODS

The study was conducted in a tertiary care referral hospital as a single center, observational study in neonates and their mothers after obtaining written informed consent from parents. The study was approved by the institute research ethics committee. All consecutively born neonates with gestation of 26 to 31 week during the study period of 1 year were enrolled. Neonates with severe perinatal asphyxia, suspected or proven chromosomal anomalies, intra-uterine infection and definite immunodeficiency in sibling or parent, and mothers with chorioamnionitis, multifetal gestation, acute febrile illness 4 weeks preceding delivery, on long-term steroids, recent vaccination and TORCH (toxoplasma, others, rubella, cytomegalovirus and herpes) infections were excluded. The gestational age of the baby was assessed from the first day of last menstrual period or first trimester ultrasonography, whichever was available. Postnatally gestational age was confirmed by New Ballard score. Demographic details, gravidity, mode of delivery, HIV (Human immunodeficiency virus) status, TORCH serology and any other infections during pregnancy were recorded for the mother. Neonatal details included birth weight, sex, APGAR and antenatal steroids. Various morbidities during the hospital stay including respiratory distress, sepsis, shock, total parenteral nutrition, neonatal jaundice, feed intolerance and hypoglycemia were recorded.

Each mother's venous blood (2 mL) was collected prior to delivery for immunoglobulin profile (IgG, IgM and IgA). Five mL of cord blood was collected for immunoglobulin profile (IgG, IgM and IgA) and lymphocyte subsets (B cells, T cells, NK cells and NK-T cells). Samples were centrifuged within 24 hours of collection and the separated serum was then stored in refrigerator at -80^{0}C and processed later for immunoglobulins. Lymphocyte subsets were assessed within 24 hours of collection. Serum IgG, IgA and IGM were estimated by endpoint nephelometry on a semi-automated nephelometer MININEPH (The Binding Site).

Lymphocyte subsets (T, B and NK, NK-T cells) were estimated using monoclonal antibodies against CD45, CD3, CD19 and CD16/56. Lymphocytes were gated using CD45 and side scatter, and the lymphocyte subsets were estimated in the gated lymphocyte population. Fifty μL of EDTA anti-coagulated blood was pipetted into a FACS tube and 10 μL

each of fluorochrome labelled CD45, CD3, CD19 and CD56/16 were added to the tube and vortexed for proper mixing of the antibodies with the test sample. The tube was then incubated in the dark for 15 minutes. Following incubation, 1 mL of lysing solution was added to the tube and incubated in the dark for 10 minutes. The tube was then centrifuged at 1200 rpm for 10 minutes, the supernatant was discarded, and the stained cell pellets were washed twice with Phosphate buffer saline (PBS) and suspended in 500 µL of PBS before sample acquisition on a flow cytometer (Navios 2 laser 6 color flow cytometer, Beckman Coulter). Following sample acquisition, data analysis was performed using the Kaluza flow cytometry data analysis software. Lymphocytes were gated on CD45 vs side scatter and the different subsets in the gated lymphocytes were estimated using dot plots and histograms.

In the absence of previous data in very preterm neonates, it was decided to enrol a convenience sample of 25 mother-infant pairs at each gestational age.

Statistical analysis: Statistical analysis was done using SPSS version 22. Comparisons were made by using student *t* test, Mann-Whitney *U* test, Chi-square test and Kruskal-Wallis test as appropriate. A *P* value of <0.05 was considered significant.

RESULTS

A total of 115 neonates and their mothers were enrolled in the study (26 to 27 weeks-18, 28 weeks-22 and 25 each from 29-31 weeks). The demographic details of the neonates are given in **Table I**. The immunoglobulin profile of neonates and their mothers are presented in **Web Tables I** and **Table II**. The lymphocyte subsets of the neonates are given in **Table III**.

The immunoglobulin profile (IgG, IgM and IgA) of mothers and neonates with and without sepsis did not differ significantly. The same was true for lymphocyte subsets in the neonates.

DISCUSSION

The present study noted increasing values of IgG and IgA with gestation. The lymphocyte subsets were not significantly different among the preterm neonates of 26 to 31 week gestation. There was no difference in immunoglobulin profile of neonates and mothers and lymphocyte subsets of

Table I Demographic and Clinical Characteristics of Preterm Neonates (*N*=115)

Variables	*26 wk (n=10)*	*27 wk (n=8)*	*28 wk (n=22)*	*29 wk (n=25)*	*30 wk (n=25)*	*31 wk (n=25)*
Birthweight (g)[a]	846 (169)	913 (121)	1076 (198)	1112 (231)	1189 (217)	1369 (264)
Male	5 (50)	5 (62)	13 (59)	10 (40)	13 (52)	11 (44)
SGA	1 (10)	0	1 (5)	6 (24)	9 (36)	8 (32)
Suspect sepsis	7 (70)	5 (63)	12 (55)	18 (72)	20 (80)	10 (40)
Proven sepsis	0	1 (13)	2 (9)	2 (8)	1 (4)	1 (4)

Values in no. (%)or [a]mean (SD). SGA-small for gestational age.

Table II Immunoglobulin Profile of Mothers of Neonates With Gestational Age of 26-31 Week (*N*=115)

Variables (in g/L)	*26 wk (n=10)*	*27 wk (n=8)*	*28 wk (n=22)*	*29 wk (n=25)*	*30 wk (n=25)*	*31 wk (n=25)*
Immunoglobulin G	7.8 (6.2-11.9)	10.8 (9-12.8)	9.1 (6.8-11.2)	8.8 (7.2-9.9)	8.8 (6.4-10.9)	7.7 (7-9)
Immunoglobulin M	0.99 (0.5-1.4)	0.92 (0.8-1.1)	1.02 (0.8-1.6)	1.2 (0.9-1.5)	1 (0.5-1.5)	1.1 (0.8-1.3)
Immunoglobulin A	1.5 (1.1-1.8)	1.9 (1.6-2.4)	1.5 (1.2-2.0)	1.7 (1.2-2.1)	1.8 (1.3-2.3)	1.7 (1.2-1.9)

Values in median (IQR). All P >0.05.

Table III Lymphocyte Subsets in Cord Blood of Neonates With Gestational Age of 26-31 Week (*N*=115)

Variables	*26 wk (n=10)*	*27 wk (n=8)*	*28 wk (n=22)*	*29 wk (n=25)*	*30 wk (n=25)*	*31 wk (n=25)*
B cell	8.3 (5.1-13.7)	12.3 (8.9-19.2)	12.2 (8.3-22.2)	12.2 (9.4-19.3)	12.6 (8.5-19.1)	12.5 (8.7-18.1)
T cell	77.9 (73.6-84.3)	74.2 (64.7-78.7)	69.9 (61.6-79.5)	71.3 (58.1-80.1)	70.9 (59.9-79.8)	75.0 (65.6-80.8)
NK cell	7.2 (5.9-9.4)	8.4 (6.4-13.5)	10.2 (6.0-14.6)	9.0 (4.5-15.8)	10.4 (6.3-18.7)	7.4 (4.2-13.2)
NK-T cell	0.9 (0.5-1.7)	0.81 (0.5-2.1)	0.49 (0.4-1.0)	0.79 (0.5-1.2)	0.98 (0.4-1.3)	0.7 (0.6-1.1)

Values presented as percentage of total lymphocytes in median (IQR). All P>0.05. NK cell – natural killer cell.

WHAT THIS STUDY ADDS?

- The normal values of immunoglobulins and lymphocyte subsets for neonates of each gestation are presented.

neonates who developed sepsis and who did not.

The immunoglobulin levels in the study were different from that by Boersma, et al. [4]. This was probably due to differences in the gestation ages of enrolled neonates, with lower gestation in present study compared to other studies. Ahmad, et al. [7] reported IgG levels in preterm in two groups (<34 weeks and >34 weeks). The IgG levels in the present study (5.34 g/L) are lower than Ahmad, et al. [7]. (6.41 g/L), the difference probably due to the differences in gestation in enrolled neonates. The study by Ozdemir, et al. [6] enrolled neonates of <28 weeks, 29-31 weeks, 32-37 weeks and ≥38 weeks. The mean (min-max) IgG levels were 3.7 (1.5-9.6) g/L, 5.4 (3.1-8.7) g/L, 6.7 (3.3-11) g/L and 7.9 (4-20) g/L, respectively. Conway, et al. [13] noted a linear correlation of IgG levels with increasing gestation. Our study also showed similar trend. Sharma, et al. [14] found mean (SD) and range of IgG, IgM and IgA values in 40 preterm infants were 11 (1.5) g/L and 9-14 g/L, 0.34 (0.06) g/L and 0.2 to 0.4 g/L, 0.02 (0.03) g/L and 0-0.12 g/L, respectively, which are higher than our study, probably because they included preterm infants of higher gestations up to 36 weeks. Panayotou, et al. [15] studied serial IgA and IgM in cord blood in healthy preterm neonates of 28-35 weeks of gestation and found that IgA was absent in most cord blood samples. This was in contrast to the presence of IgA in cord blood samples in the present study. As IgM does not cross placenta and hence high cord IgM may reflect underlying intrauterine infection however we had very low value of IgM in our study.

When comparing lymphocyte subsets, there were no studies that had separate values at each gestation. Berrington, et al. [8], observed that preterm had 65% T cells, 23% B cells and 7% NK cells in their blood. These were nearly similar to our study. The T cells and B cells in the study by Quinello, et al. [9] in gestation 30 weeks to 33 weeks were 43.3% and 16.1%, respectively. Our study showed T cells and B cells to be 70.8% and 13.9%, respectively at gestation 30 to 31 weeks. Our study also found no difference in lymphocyte subsets in those who developed sepsis and those who did not. This is in contrast to the study by Bochennek, et al. [12], which showed that neonates who developed sepsis had significantly lower NK cells. The cause for this could not be evaluated.

This study showed that there was no statistically significant difference in the immunoglobulin profile and lymphocyte subsets of the preterm neonates with regards to occurrence of sepsis. However, this study had a small sample size and further studies are needed to establish the normal values of immunoglobulins and lymphocyte subsets at each gestation and their relation to occurrence of sepsis.

Ethics clearance: Approved by the institutional ethics committee no. NK/3996/MD; dated June 28, 2019.
Contributors: KM, RS: conceived the idea of this study; RS, KM, AR, VS, SS: involved in formulating the protocol of this study; RS: collected the samples for this study. The samples were processed in the laboratory under guidance of AR and SS. RS, KM, AR, VS, SS were involved in the writing of this manuscript. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.
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Note: Additional material related to this study is available with the online version at *www.indianpediatrics.net*

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CLIPPINGS

Age adjusted N-Terminal-proBNP (NT-proBNP) to predict major adverse cardiovascular events (MACE) in children *(J Am Coll Cardiol. 2021;78:1890–1900)*

Despite being a valuable prognostic biomarker, NT-proBNP is not established as a prognostic biomarker in pediatric cardiac diseases due to strong age-dependency of its value. In this study, including 910 children with congenital heart disease (CHD), zlog values of NT-proBNP were utilized for age independent evaluation to determine its prognostic power for major adverse cardiovascular events (MACE) (death, resuscitation, mechanical circulatory support, or hospitalization due to cardiac decompensation) in children with CHD. During a median follow up period of 6 months, MACE occurred in 138 children. High zlog NT-proBNP values (>+3.0) were strongly associated with adverse events (adjusted HR 21.1; 95% CI 2.9-154.2, P<0.001). A cut off value of +1.96 achieved a negative predictive value of >96%. Hence, zlog NT-proBNP may play an important role in future management of children with heart disease.

2021 PACES Expert Consensus Statement on the indications and management of cardiovascular implantable electronic devices (cieds) in pediatric patients *(Cardiol Young. 2021;31:1738–69)*

Disease substrates and indications for cardiac implantable devices differ widely among pediatric and adult patients. Therefore, adult guidelines cannot be extended to pediatric population. In 2021, Pediatric and congenital electrophysiology society has released expert consensus statement on the indications and management of cardiovascular implantable electronic devices for appropriate use in pediatric patients. These include indications and management of permanent pacemaker in congenital and acquired heart block; implantable cardioverter defibrillators and insertable cardiac monitors for patients <=21 yr age.

Unification of clinical and administrative nomenclature for pediatric and congenital cardiac care– International Pediatric and Congenital Cardiac Code (IPCCC)-2021 and ICD-11 *(Cardiol Young. 2021;31:1057-1188)*

Development of classification schemes specific for congenitally malformed hearts began with Dr Abott's atlas in 1936. Over the years various attempts have been made to classify the congenital heart diseases and currently International Pediatric and Congenital Cardiac Code (IPCCC) has been incorporated as such in the eleventh revision of International Classification of Diseases (ICD-11). The total number of pediatric and congenital cardiac terms in ICD-11 is 367. This global system of nomenclature for pediatric and congenital cardiac care unifies clinical and administrative nomenclature.

Outcome of COVID-19-positive children with heart disease: A Multi-centric Study from India (*Ann Pediatr Card. 2021;14:269-77)*

From pediatric cardiac centers across India, authors retrospectively studied 94 children and grown-ups with congenital heart disease. One third of patients were symptomatic for COVID-19 and the remaining were incidentally detected positive on screening. Overall mortality in the cohort was 13%. Among the patients who required admission mortality rate was 28%. The risk factors for mortality were disease severity at admission and low socio-economic status.

AHA scientific statement for treatment of myocarditis in children *(Circulation. 2021;144:e123-135)*

Myocarditis in children is a challenging disease and its diagnostic workup, management and follow up are complex with not much evidence. In this scientific statement, authors have defined myocarditis into four strata as biopsy proven, cardiac magnetic resonance (CMR)-confirmed clinically suspected, clinically suspected, and possible myocarditis. The writing group has comprehensively mentioned the current evidence for the role of various investigations and management including medical stabilization and interventions like ECMO, ventricular assist devices and cardiac transplant.

Efficacy and safety of propranolol in infants with heart failure due to moderate-to-large VSD *(Ann Pediatr Card 2021;14:331-40)*

This randomized controlled trial aimed to assess the efficacy and safety of propranolol in 80 infants with heart failure due to moderate to large ventricular septal defect. The primary end-point was the composite all-cause mortality, hospitalization for heart failure, and/or chest infection and referral for surgery. Propranolol in addition to conventional therapy significantly decreased the risk of hospitalization and worsening of Ross heart failure class. There was a trend towards improvement in the primary composite end-point. Therapy was tolerated well without any significant side effects.

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