MATERIAL AND METHODS
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This prospective study was conducted in the Department of Pediatrics, M.L.B. Medical College and Allied Hospital, Jhansi, in active collaboration with the Department of Obstetrics and Gynaecology, over a period of one year from May 1990 to April 1991. The cases included in the study, were selected from the newborns delivered in the hospital and those admitted in the Pediatric ward of the M.L.B. Medical College and Allied Hospital, Jhansi. All newborn babies were broadly divided into four sub-groups for assessment of their immunological profile.

1. Normal full term healthy newborn babies (Control).

2. Low birth weight infants -
   (a) Appropriate for gestational age (AGA) premature infants.
   (b) Small for date (SFD) babies which included mature as well as premature babies.

3. Infants with neonatal hyperbilirubinemia.

4. Infants with neonatal infections.
SELECTION OF CASES

Cases were selected in different sub-groups according to the following selection criteria.

Control: Twenty full term healthy newborns served as control for the present study. Care was taken to exclude all those factors which could adversely affect the immunological status of these newborns. The criteria of selection of these cases was -

1. Weight above 2500 gm.
2. Gestational age ranging from 37 to 41 weeks.
3. Apgar score at the time of delivery varying from 7 to 10.
4. There was no history of infection, toxaemia, diabetes, prolonged rupture of membranes in the mother during pregnancy and labour.
5. None of the newborns were suffering from any infection or congenital malformation.

Blood samples were taken from the umbilical cord in all the control cases at the time of birth.

Low birth-weight babies:

Twenty low birth weight newborns were taken for the present study. These included ten appropriate for
gestational age premature babies, and ten small for date (S.F.D.) infants, having weight less than 10th percentile for gestational age. The gestational age was assessed by the date of last menstrual period, and by physical characteristics given by Usher's criteria (1965). The criteria for selection of these cases was -

1. All the premature babies had gestational age below 37 weeks.

2. The S.F.D. babies were those showing features of intra-uterine malnutrition evidenced by features of decreased linearity, loss of subcutaneous fat, loose dry skin and sparse hairs (Lubchencar, et al, 1963; Naeye, 1966; Drillen, 1970 and Usher, 1970).

3. All the low birth weight babies (Premature and S.F.D.) were product of normal vaginal delivery and none of the newborns had any evidence of infection.

In both the group of cases blood sample was obtained from the umblical cord at the time of birth. Those samples not fulfilling the criteria of selection of low birth weight were discarded.

SELECTION OF CASES OF NEONATAL HYPERBILIRUBINEMIA:

Only those newborns were included in the study who were having a serum bilirubin level of 10 mg per 100 ml
Twenty full term normal weight newborns having plasma bilirubin levels ranging from 10.8 to 30 mg per 100 ml were included in the present study. Samples were taken from femoral vein in all the cases within a period of one week after birth. Care was taken to exclude those cases of neonatal hyperbilirubin with deep infection. Out of these 20 neonates having hyperbilirubinemia, 14 were having prolongation of Jaundice due to umbilical sepsis, 4 had Rh incompatibility while the remaining 2 had physiological jaundice.

**SELECTION OF CASES OF NEONATAL INFECTION:**

Twenty newborns suffering from various infections were taken into consideration for this study. Six were having umbilical sepsis, another six were having pyogenic meningitis, four had pneumonitis and remaining four had neonatal septicemia with multiple pyemic abscesses. The criteria for the selection of these cases was failure to suck, hypo or hyperthermia, episodes of cyanosis, convulsions and other systemic manifestations. Samples were obtained in all the cases from femoral vein within a period of one week.

**Antenatal and Natal history -**

A complete antenatal history pertaining to drug intake, irradiation, infections and systemic disease in the mother was taken into account. Natal history with
regard to rupture of membranes \(< 12\) hours or \(\geq 12\) hours, and mode of delivery was recorded in each case. The immediate post-natal history pertaining to apgar score, cry after birth and colour was noted in each case.

**Examination of Newborn** -

In all the newborns a detailed examination was done to detect any systemic disease. Examination of the baby was done in great detail with special reference to appearance, colour, cry, activity, cyanosis, jaundice, anaemia, any congenital malformation in the baby. Detailed examination was done to find out any source of infection in the form of septic focus or umbilical sepsis or any other infection. Posture, reflexes, sucking was also noted in each case. Due emphasis was given to note the anthropometric measurements i.e. weight, length, chest and head circumference in each case. The gestational age was assessed in each case by the physical criteria of assessment as well as by the date of last menstrual period.

**Investigation** -

Diagnosis of type of neonatal hyperbilirubinemia was done by finding the level of total, direct and indirect bilirubin in the serum.

Cases of neonatal infection were confirmed by doing the total leucocyte count and differential leucocyte
count. X-ray chest and culture from local septic focus was done whenever needed. Diagnosis was confirmed by blood culture wherever needed.

Necessary investigations viz. blood counts, G.B.P., serum bilirubin, reticulocyte count, Coombs test, blood culture, blood grouping, X-ray chest, C.S.F. examination and smear (gram staining) were carried out wherever needed.

Collection of sample:

Blood samples for the present study were taken either from the umbilical cord (in controls and low birth weights) at the time of delivery or from the femoral vein (in hyperbilirubinemia group and infection group). 5 ml sample of blood was collected from each case in a plain vial and serum was separated and was stored at -20°C for determination of immunological profile.

METHODS: Following immunological investigations were done in each case.

1. Serum immunoglobulin IgG & IgM.

2. Complement C3 levels.

Immunoglobulin and complement determinations were done by the method of single radial diffusion of Mancini et al (1965). Solugen (R) S.R.I.D., Ready to use immunoplates of each immunoglobulin supplied by M/S Immuno-
diagnostics Pvt. Ltd. were utilized. The blood samples taken from the cord at the time of birth or from femoral vein were centrifuged, sera was separated immediately by kept in deep freeze at -20°C, till the time of immunoglobulin determination.

Procedure:

Each immunoglobulin plate has 12 wells in which the different dilutions of standard reference sera and the serum samples were filled with the help of a capillary tube and care was taken not to underfill or over fill the wells. For accurate determination of IgG the patients serum was diluted five times with isotonic saline (1 part serum : 4 part saline), while no dilution was however done for estimation of IgM and complement C₃. After the wells were filled, the lid of the plate was replaced and the plate was left for the development of the precipitin ring in inverted position for 24 hours at room temperature. In case of estimation of IgM, plates were incubated at 4°C for another 24 hours. The ring diameters were measured by an immunomeasure and standard graph for each immunoglobulin were constructed using the values of the "Reference standard". The diameter ($d^2$) were plotted on the (↑) ordinate while the quantitative value (Reference standard values) were plotted on the (→) abscissa of the graph. Thereafter the values of the unknown samples were found out directly by interpolation and extrapolation on the standard graph. Results were expressed in mg/100 ml.