MATERIAL AND METHODS
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The present study was carried out in the Department of Paediatrics, in active collaboration with the Department of Obstetrics and Gynaecology, Maharani Laxmi Bai Medical College, Jhansi. Babies delivered in the labour room of Obstetrics and Gynaecology department, between May, 1990 to June, 1991 were included in the study.

SELECTION OF CASES

Study Group:

This consisted of 24 live newborn babies delivered by normal vaginal route. They were born at full term gestation; were singleton; had birth weight not exceeding 2500 grams. Babies born to mothers with short stature (height below 145 cm and weight below 40 Kg), gross anaemia (Haemoglobin \( < 8 \text{ gm\%} \) by Sahli's method), edema, hypertension (blood pressure exceeding 140 mmHg systolic and or 90 mmHg diastolic), congestive cardiac failure, evidence of perinatal stress, metabolic disease and blood group incompatibility were excluded. Also, babies born to mothers with a history of febrile episode in the third trimester and those having early rupture of membranes were excluded from the study.
Control Group:

This consisted of 10 healthy live newborn babies delivered by normal vaginal route. They were born at full term gestation; were singleton; had birth weight 3000 gms or more.

Obstetrical History:

In each case, history of last menstrual period was recorded when the mother was sure of it. Gestational age was calculated in complete weeks from the first day of last menstrual period up to the time of delivery.

Natal and Postnatal History:

History was taken to record the mode of delivery. The other points noted were duration of labour, per vaginal bleeding, meconium staining of liquor, cry and activity of the child after birth including cyanosis; all to rule out any evidence of perinatal stress. All cases presenting with perinatal stress were excluded from the study.

Examination of Newborn:

Apgar scoring of the child was done at 1 minute and after 5 minutes following birth to detect any evidence of birth anoxia. Cases having Apgar score less than 7 were excluded from the study group.
Thorough clinical examination was done in each case to exclude the possibility of congenital infection or anomaly. Anthropometric measurements viz., head circumference, chest circumference, crown-heel length were recorded in the proforma. Birth weight of the newborn was recorded within one hour of the delivery.

Assessment of gestational age was done by using the physical and neurological characteristics laid down by Dubowitz et al (1970). The neurological characteristics were scored from 0-5, while eleven physical characteristics were scored from 0-4 in a pre-designed proforma and conversion of total score into gestational age was done by using the formula (Dubowitz et al, 1970).

Estimated period of gestation = \( R \times 0.2642 + 24.5950 \) (in weeks)

Where \( R \) represent the total score.

In the case of discrepancy found between gestational age calculated by Dubowitz criteria and that calculation from the history of menstrual period, the case was dropped from the study.

Collection of blood sample:

For the estimation of Ig M, blood (5 ml) was collected from the cut end of umbilical cord, from the placental side, in the clean glass tube, with due precautions to avoid haemolysis and contamination with maternal blood. All glassware
used in the study was thoroughly sterilized, washed with distilled water and dried in hot air oven.

Blood sample was allowed to clot at room temperature. After 2-4 hours, serum was separated using a pipette and then serum was centrifuged at 1000 rpm for 15-20 minutes. After centrifugation 2 ml of clear serum at the top of sample was transferred to another dried vial. All samples were stored at -20°C in a deep freezer. Similarly, a venous blood sample of the mothers was taken and serum was separated and stored in the deep freezer. The samples were analyzed for Ig M at a later date.

Method of Estimation:

Serum immunoglobulin Ig M was determined by using single radial Immunodiffusion method (Mancini et al, 1965). For this commercially prepared Immunodiffusion plates were used. The principle being that specific antibody against human immunoglobulin is incorporated into a buffered agarose medium and uniformly spread on a glass plate. The test serum containing the human immunoglobulin (antigen) is placed in a well prepared in the antibody agarose plate. When agarose contains sufficient amount of antibody, there is a free diffusion of antigen from the well. Reference serum containing known amount of immunoglobulin (antigen) is run concurrently with unknown specimens. Since the immunoglobulin concentration
in the serum is related to the area of precipitin zone, 
this can be directly read from the table of the reference 
value or from the calibration curve.

(i) Reagents and Material used:
1. Immuno diffusion plates
2. Reference serum
3. Auto pipette
4. Measuring scale for measuring the diameter of 
precipitin ring.

(ii) Preparation of Test Sera and Reference Serum Dilutions:

For obtaining accurate results, the reference serum 
and test serum were diluted, using 0.85% normal saline 
as shown in Table - I.

<table>
<thead>
<tr>
<th>Dilution required for</th>
<th>Reference Serum</th>
<th>Test Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100% 75% 50% 25%</td>
<td>50%</td>
</tr>
<tr>
<td>Undiluted</td>
<td>3/4 part</td>
<td>1/2 part</td>
</tr>
<tr>
<td>Reference Serum and</td>
<td>1/4 part</td>
<td>1/4 part</td>
</tr>
<tr>
<td>1/4 part</td>
<td>1/2 part</td>
<td>1/2 part</td>
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<tr>
<td>Normal saline</td>
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<td>Normal saline</td>
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</tbody>
</table>

TABLE - I
(iii) Procedure:

1. Lid of the Immuno-diffusion plate (having 16 wells) was carefully removed. Each of the first four wells was filled with 5 microlitres of the reference serum of different dilutions (100%, 75%, 50%, 25%) and the remaining wells were filled with 5 microlitres of the test sera appropriately diluted as mentioned in the Table - I.

2. Lid of the plate was then replaced and the same was incubated at room temperature (25-30°C) for 72 hours.

3. After incubation, the diameter of each precipitin ring was measured accurately (upto 0.1 mm accuracy) with the help of a measuring scale.

4. The Ig M concentration corresponding to the measured diameter of precipitin ring was read directly from the calibration curve.

(iv) Calibration Curve:

Calibration curve was drawn using squared diameter of the precipitin rings obtained from various dilutions (25%, 50%, 75% and 100%) of the reference serum. On a centimeter graph paper, the squared diameter of precipitin rings were plotted on the vertical axis (Y-axis) against the corresponding known immunoglobulin concentrations (mg per 100 ml)
on the horizontal axis (X-axis). Using 4 points of square of diameter against dilutions of reference serum concentration, a straight line of best fit was drawn. The concentration of particular immunoglobulin in the test serum was determined by reading the concentration against the point representing the square of the diameter of the respective ring of precipitate.