MATERIAL AND METHOD
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The present study was carried out in the Department of Paediatrics, P.L.B. Medical College, Jhansi, over a period of one year from June 1988 to June 1989. Neonates from birth to one month of age admitted from the Out Patient Department of Paediatrics and admitted in the Paediatrics ward, as well as babies delivered in the Department of Obstetrics and Gynaecology were selected for the present study. The clinical history and the findings of physical examination were recorded on a pre-designed proforma.

Our case material was grouped as -

A. Control - 10 normal, full term and pre-term babies were taken as control for the present study.

B. Study groups -

I. Cases showing evidence of superficial infections (umblical sepsis, conjunctivitis, impetigo etc.).

II. Cases showing evidence of deep infections (septicemia, pneumonia, meningitis etc.).

SELECTION OF CASES -

A. Control - Total 10 cases were taken as control for the present study. Antenatal, natal & post-natal history
was normal in all the cases, viz. maternal infection, foetal asphyxia, foetal distress and prolong rupture of membrane. All the cases were delivered by normal vaginal route. Out of 10 cases, 5 cases were full term normal babies while 5 cases were pre-term. All the babies had no evidence of any superficial or deep infection.

B. Study Group -

I. Selection of cases of superficial infections -

The selection of cases of neonatal superficial infection was based on a significant history of pus discharge from umbilicus, pus discharge from eyes, sticking of eyes, any superficial boils or vesicles over the body.

II. Selection of cases of deep infections -

The selection of cases of deep infection was based on the history of failure to suck, excessive cry, letharginess, jaundice, GIT manifestations in the form of diarrhoea and vomiting, bouts of hypo or hyperthermia. The diagnosis was based on thorough clinical examination, history and laboratory tests. Besides blood routine, urine routine, X-ray chest, cytobiochemical examination of CSF, blood culture and sensitivity was also done to find out evidence of any deep infection.
Antenatal/Natal/Post-natal History:

A detailed account of history was taken in each case. Special emphasis was given to obtain the history of antenatal period i.e., history of taking drugs, irradiations and viral infections. Subsequently in the 2nd and 3rd trimester, due emphasis was given to elicit the history of systemic infections with special reference to diabetes, hypertension, toxemia of pregnancy and antepartum haemorrhage. The type of delivery normal or abnormal (forceps application or lower segment cesarean section) was elicited in each case. In the natal period, due emphasis was also given to the history of leaking of more than or less than 12 hours, delivery conducted by trained personnel or untrained personnel, repeated vaginal examinations prior to delivery and hospital or home delivery. The assessment of gestational age was done by the history of last menstrual period as well as by the physical characteristics given by Usher et al.

Clinical Examination:

A thorough clinical examination was done in each case. General physical examination, included a general appearance of the baby, colour, cry, activity and cyanosis. Congenital anomalies were also looked for in each case. Gestational age assessment was done in each case by the morphological features given by Usher et al. Anthropometric measurements were recorded in each case and due emphasis
was given to assess the birth weight and head circumference in each case. Jaundice and transient skin manifestations viz. cutis marmorata, erythema toxicum and colour changes due to instability of vasomotor centres, umbilical sepals and furunculosis were looked for in each case. The eyes were looked for any evidence of jaundice, conjunctivitis sub-conjunctival haemorrhage and dehydration.

In each case a thorough systemic examination was done. The level of consciousness, cranial nerves, posture of the baby, superficial as well as deep tendon reflexes were examined in detail. Due emphasis was given to assess the important neonatal reflexes and the size and pulsations of the anterior fontanelle. The cardiovascular system, respiratory system and abdominal system were also assessed in detail in each case to look for evidence of lung infections, GIT problems and any evidence of congenital heart disease.

**Investigations**

Laboratory investigations, viz. haemoglobin, total and differential leucocyte count was done in each case. CSF examination for colour, coagulum, protein, sugar, chloride and cells were carried out in cases of septic meningitis, while in neonatal septicemia, blood culture and sensitivity was done in each case. Special investigations like stool, urine, pus, conjunctival swab culture and
sensitivity were done as and when required. In cases of pneumonia, X-ray chest was done. X-ray of the relative parts were done in cases of septic arthritis.

**Estimation of CRP in serum:**

For quantitative estimation of serum CRP, single radial immuno-diffusion technique as described by Kancini et al (1965) was used.

The principle of this technique is "antigen diffuses radially from the points of application into an antibody containing gel and a circular precipitate or ring forms at the zone of equivalence keeping antibody concentration and gel thickness constant the area covered by precipitation ring is proportional to the concentration of antigen".

The blood was taken on the first day of admission before starting the antibiotics in all the infected cases. Serum CRP values were estimated by single radial immuno-diffusion technique, kit manufactured by M/s Immuno-diagnostic Pvt. Ltd.

**Procedure -**

Two or 3 ml of blood was aseptically collected from the peripheral vein of each patient of the study and control group. The sera was separated by centrifugation at 3000 rpm for 10 minutes and was stored at -20°C.
- Serum sample were brought to room temperature.

- For the estimation of CRP level, the wells were charged with micropipette without overflow outside the wells.

- The plates were incubated in a moist chamber at room temperature for 48 hours and examined under oblique illumination for the presence of precipitin rings.

- Control sera was also charged in the wells with micropipette.

- Reference sera in the dilutions 1:1, 1:2 and 1:4 were also charged in the wells.

Calculations -

The size of the precipitin rings were recorded using a trichotigen scale. The quantity of CRP in each sample was calculated using $d^2$ on a standard curve. For the preparation of standard curve of CRP, the wells were charged with dilutions (1:1, 1:2 and 1:4) of standard serum containing known amount of CRP (70 ug/ml). The diameter of precipitin rings were recorded and a curve was plotted using the square of diameter ($d^2$) and the known concentration of CRP in standard control serum.
<table>
<thead>
<tr>
<th>Reference serum dilution</th>
<th>Max. concentration of CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 : 5 (100%)</td>
<td>70 ug/ml</td>
</tr>
<tr>
<td>1 : 1 (50%)</td>
<td>35 ug/ml</td>
</tr>
<tr>
<td>1 : 2 (25%)</td>
<td>17.5 ug/ml</td>
</tr>
<tr>
<td>1 : 4 (12.5%)</td>
<td>8.8 ug/ml</td>
</tr>
</tbody>
</table>

Statistical analysis was done to derive mean and standard deviation (S.D.). Mean values were compared using 't' test and significance of difference was tested.
Diameter in millimeter ($D^2$)

Standard curve of C-reactive protein

100% = 2991.09
50% = 1376.09
25% = 625.00