MATERIAL
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METHOD
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The present study was conducted in the Department of Paediatrics, M.L.B. Medical College, Jhansi over a period of one year from July, 1992 to June, 1993. Neonates from birth to one month of age delivered in Department of Obstetrics & Gynaecology and those directly admitted in the Department of Paediatrics were included for the present study.

A total of 62 cases were included for the study.

The case material was broadly divided into -

1. CONTROL GROUP:

It was further subdivided into -

(a) Full term:

10 full term normal weight healthy neonates born normally to healthy mothers without any risk factor for neonatal sepsis were included in this group.

(b) Preterm:

5 preterm healthy babies were selected as preterm control. Care was taken to include only those babies whose mother had no risk factor for development of infection. These babies also had not any evidence of infection on follow up.
2. **STUDY GROUP**

   It was subdivided into three groups -

   (a) **Proved Sepsis**:

   14 neonates with clinical features of neonatal sepsis and having positive blood culture - designated as group A.

   (b) **Probable sepsis**:

   14 neonates, clinically showing features of septicemia but in which blood culture was negative - designated as group B.

   (c) **Superficial infection**:

   18 cases showing evidence of superficial infection like umblical sepsis, conjunctivitis, pyoderma etc - designated as group C.

**SELECTION OF CASES**

**Control group**:

10 full term and 5 premature babies were taken as control. Antenatal, natal and postnatal history was normal in each case. All the babies of this group were delivered normally by vaginal route. History of prolonged rupture of membrane, maternal infection, birth anoxia, fetal distress and other high risk factor were excluded.
STUDY GROUP:

Selection of suspicious cases of sepsis (Proven & Probable):

This group comprised of 28 clinically suspected cases of neonatal septicemia. The criteria of selection of these cases were failure to suck, lethargy, irritability, hypo or hyperthermia, diarrhoea, vomiting and other subtle signs of infection.

A detailed obstetrical history was taken in each case after admission.

Antenatal History:

A detailed history of infection (particularly in first trimester), systemic disease of mother viz diseases related to respiratory, cardiovascular, metabolic and neurological systems were taken in each and every cases. Due consideration was also given to elicit the history of APH, eclampsia, toxemia, ABC, Rh incompatibility in each case. Special emphasis was also given to elicit the history of leaking P/v as well as colour and odour of amniotic fluid.

History of number of vaginal examinations were also recorded in each and every case.
Natal History:

Due emphasis was given to elicit the mode and presentation of deliveries in each case viz by normal vaginal route or abnormally by forceps or caesarean section. Apgar score at one minute and five minute was also recorded in hospital deliveries.

Postnatal History:

History of resuscitation, hyperthermia or hypothermia, cyanosis, irritability, refusal of feeds, lethargy, convulsion, abdominal distension, prolongation of jaundice, diarrhoea, vomiting etc, was also recorded in each and every case.

Examination of Newborn:

A detailed general and systemic examination of the newborn was done in each and every case. Accordingly colour, cry, activity and posture was noted. Anthropometric measurements viz, weight, head circumference, chest circumference and length of baby were also recorded.

Gestational age was assessed by Usher's physical characteristics criteria viz, texture of hairs, ear cartilage and recoiling, size of breast nodule, scrotal rugae or position of labia majora and creases over the sole.
Examination of head for caput or cephalhaematoma, palpation of anterior fontanelle and sutures, examination of face, oral cavity, neck and trunk was done in each case. Due emphasis was given to observe colour of umbilical cord (meconium stained or not) jaundice and cyanosis.

Life threatening congenital anomalies and other congenital anomalies viz, choanal atresia, tracheo oesophageal fistula, meningocele, meningomyelocele, tumour in the neck, features of Down's syndrome, congenital heart disease and renal malformations etc were also recorded.

Systemic examination:

Cardiovascular system, respiratory system, examination of abdomen and complete neurological examination was done in all the newborn babies. Special emphasis was given to note the character of first and second heart sound, murmur and sign of congestive heart failure. Enlargement of liver, spleen, kidney, or any lump in abdomen was also noted. Signs of respiratory distress viz, respiratory rate more than 60/min. in quiet respiration, grunting and retraction was noted. Percussion and auscultation of the chest was done to exclude any respiratory problem. A detailed neurological examination was performed and an effort was made to elicit the important neonatal reflexes to assess the neurological status of newborn.
Selection of cases with superficial infection:

The selection of cases for this group was based on significant history of pus discharge from umbilicus, sticky eyes and pus discharge from eye. Any superficial vesicle, pustule, boil or abscess was also looked over skin.

METHOD:

1. Each case was subjected to a detailed clinical history and a thorough physical examination.

2. The following investigations were undertaken -

(A) Bacteriological Investigations:

Blood Culture:

Blood culture was sent immediately after admission in every suspected case of neonatal sepsis before start of antibiotics. One ml of blood collected from peripheral vein by disposable syringe and needle under full aseptic precaution was inoculated in 10 ml of glucose citrate broth in screw capped bottle. This was incubated at 37°C for 18 to 48 hours. If the medium showed any turbidity, it was subcultured on blood agar or MC Conkey's agar plate and smear was prepared and examined. The bacteria thus isolated were identified and tested for their antimicrobial drug sensitivity.
Fus culture:

Fus swab from conjunctiva, umbilicus, vesicle pustule etc. were also sent for culture and sensitivity and for identification of microorganism.

(B) MICROCERYTHROCYTE SEDIMENTATION RATE (m ESR):

Principle:

The basis principle underlying this technique is same as that of measurement of ESR by conventional method (Westergren's method) i.e., the anticoagulated blood is allowed to stand vertically in a glass tube of stand length and bore size at room temperature and the reading is taken at the end of one hour.

Procedure:

For measurement of m ESR, heparinised capillary tube of 75 mm of length, internal diameter of 1.1 mm and outer diameter of 1.5 mm manufactured by TCP SYRINGES, BOMBAY was used.

The capillary tube was filled rapidly and completely with capillary blood obtained by heal prick. Air was not allowed to interrupt the column of the blood. One end of capillary was sealed with plasticin.
The tube was then mounted vertically with the help of an adhesive plaster on the wall. At the end of one hour the distance from the top of the tube to the meniscus of the packed red cell column was measured. Value was expressed as mm/first hour (Adler & Denton 1975).

(C) **ESTIMATION OF SERUM C REACTIVE PROTEIN** :

In our study quantitative estimation of serum C reactive protein was done by method described by Phillips (1987) using latex agglutination method. Commercial kits supplied by SPAN Diagnostics were used.

Principle :

The CRP latex test is based upon the immunological agglutination reaction between C reactive protein present in serum and corresponding antibody coated on the surface of biologically inert latex particle.

Procedure :
1. All reagents as well as sera were allowed to reach room temperature.

2. One drop of test serum was placed within circled area on the special slide by disposable dropper provided in kit.
3. One drop of latex CRP reagent was added to above drop and mixed well with disposable applicator stick provided in kit and it was spread out to the edge of test area.

4. It was then gently rocked to and fro for two minutes and examined for macroscopic agglutination.

**Interpretation:**

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<th>Observation</th>
<th>Conclusion</th>
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<tr>
<td>i)</td>
<td>Coarse agglutination</td>
<td>Strongly positive</td>
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<tr>
<td>ii)</td>
<td>Finer agglutination</td>
<td>Weakly positive</td>
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<td>iii)</td>
<td>Smooth suspension</td>
<td>Negative</td>
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(D) **DETERMINATION OF LEUCOCYTE ALKALINE PHOSPHATASE (LAP) ACTIVITY:**

In our study cytochemical method described by Sirola & Sirola (1957) is used to determine LAP activity in polymorphs.

**Principle:**

Phosphate ions are liberated from glycero-phosphate by phosphatase contained in the cell, and in combination with these ions calcium chloride forms calcium phosphate. Cobalt nitrate together
with calcium phosphate forms cobalt phosphate. The latter finally is precipitated with ammonium sulphide as cobalt sulphide, which is visible inside the cells.

Reagents:

1. Fixative:
   (a) Cold acetone at 4°C
   (b) 95% ethyl alcohol

2. Incubating solution:
   (a) Sodium beta glycerophosphate as 5% solution.
   (b) Calcium chloride as 2% solution
   (c) Magnesium sulphate as 2% solution
   (d) Sodium diethyl barbiturate as 2% solution.
   (e) Cobalt Nitrate as 2% solution
   (f) Yellow ammonium sulphide as 2% solution
   (g) Counterstain saffranin as 2% solution.

Procedure:

1. Freshly prepared blood smear were dried in the air.
2. They were fixed within half an hour of preparation in cold acetone at 3°C for three hours.

3. They were again fixed in 95% ethyl alcohol for few minute.

4. The slides were then washed in running water and kept in incubating solution.

5. Incubating solution: 50 ml of sodium beta glycerophosphate, 10 ml of calcium chloride, 50 ml of sodium diethyl barbiturate and 2 ml of magnesium sulphate, each of 2% strength were mixed and blood smear were incubated in this solution for two hours at 37°C room temperature.

6. Then blood smear were washed thoroughly in running water and placed in 2% cobolt nitrate solution for five minutes.

7. The smear after washing again in water were kept for few minutes in yellow ammonium sulphide solution.

8. The smear were then well rinsed in water and then counterstained with saffranin for few seconds.

9. After thorough washing in running water, the smear were examined under oil immersion lens of microscope.
**Interpretation:**

In the phosphatase positive leucocyte, phosphatase granules containing cobalt sulphide were visualised with varying intensity of colour from light brown to dark brown. The phosphatase negative polymorphs were visible only as dim shadows. The cells were scored according to the intensity of the staining and individual cells were rated according to scoring system of Kaplow (1955) as -

**Score - 0** : Colourless cytoplasm

**Score - 1** : Barely visible or diffuse staining of cytoplasm with occasional granules.

**Score - 2** : Diffuse staining of cytoplasm with moderate granules.

**Score - 3** : More staining of cytoplasm with numerous granules.

**Score - 4** : Very strong staining of cytoplasm with numerous granules.

One hundred consecutive neutrophil were examined in each case and rated 0-4 accordingly. A total of these rating in hundred cells provided the final score of a particular smear.
(E) **INVESTIGATIONS**:

The following investigations were also performed wherever thought relevant.

(i) **Haematological investigations**:

TLC, DLC, Hb. Absolute neutrophil count, platelet count, toxic granules in polymorphs.

(ii) **Urine examination**:

Routine - albumin, sugar, microscopic examination and culture and sensitivity was done, wherever needed.

(iii) **Cerebrospinal fluid examination**:

Cytological, biochemical, bacteriological examination, Gram's staining and culture and sensitivity was also done in cases of pyogenic meningitis.

(iv) **X-ray chest**:

X-ray chest was done in cases suspected of having pneumonitis.

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