MATERIAL AND METHODS
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The present study was conducted in the Department of Paediatrics and Department of Microbiology, M.L.B. Medical College, Jhansi, over a period of one year from July 1992 to July 1993. One hundred cases were included in the study, out of which 75 were clinically suspected cases of neonatal septicaemia and 25 cases were healthy neonates who served as control.

Selection of cases:

All clinically suspected cases of neonatal septicaemia admitted in the Department of Paediatrics and newborns delivered in the Department of Obstetrics and Gynaecology constituted the subjects for the study. These cases were grouped under the following categories:

Category A: All clinically suspected cases of neonatal septicaemia having blood culture positive for bacteria.

Category B: All clinically suspected cases of neonatal septicaemia having blood culture negative for bacteria.

Category C: Healthy neonates who served as control.
All neonates were screened by detailed clinical history and thorough physical examination as described by Gotoff and Behrman (1970).

**History:**

A detailed history was taken with special emphasis on low birth weight, lethargy, not doing well, poor feeding, amniotic fluid infection, e.g. foul smelling, cloudy or purulent amniotic fluid, premature rupture of membranes (7-12 hr.), resuscitation problem such as intubation and umbilical vessel catheterization. Bloody secretions of upper air way suggesting aspiration of maternal blood or vaginal secretion, fever, hypothermia sclerema and or manifestations pertaining to gastro-intestinal tract, respiratory system, cerebro-vascular system, central nervous system or haematological system.

**Examination of Newborn:**

A detailed general and systemic examination was done in each and every case. Accordingly colour, cry, activity and posture of the newborn was noted.

**CNS Examination:**

Special attention was paid to note lethargy, hyporeflexia, irregular respiration, tremors, convulsions, irritability, fullness of fontanelle, opisthotonic attacks, if any.
Efforts were made to elicit the important neonatal reflexes to assess the neurological status of the newborn.

**Respiratory system:**

Tachypnea, dyspnea, cyanosis, apnea were noted.

**Gastro-intestinal system:**

Abdominal distension, hepatomegally, if any, was noted.

**Haematological system:**

Jaundice, splenomegally, pallor, purpura, petichae or any other signs of bleeding manifestations were noted, if present.

**Circulatory system:**

Cyanosis, mottling hypotherma, abnormal respiration (apnea, tachypnea, irregular respiration etc.), if present, was noted.

**Associated focal infections** were especially looked for, viz., meningitis, pneumonia, urinary tract infection, omphalitis, conjunctivitis, abscess of skin or soft tissue, impetigo, otitis media, septic arthritis, osteomyelitis, peritonitis, vaginitis, infected cephalhaematoma.
Investigations:

Following investigations were done in each case.

1. Blood culture,
2. Total leukocyte count by Neubaur counting chamber.
3. Differential leukocyte count.
4. Band cell count.
5. Band cell/total neutrophil ratio.
6. Micro ESR.

In addition to above relevant investigations, other investigations like - X-ray chest, cerebro-spinal fluid examination and urine examination etc. were done depending upon the specific need.

Laboratory Procedure:

Blood culture was done at the Department of Microbiology of M.L.B. Medical College.

Rest of the investigations were done in the Immunology and Biochemical Laboratory of the Department of Paediatrics, M.L.B. Medical College, Jhansi.

Blood Culture:

One ml of blood was collected aseptically from femoral venipuncture and was transferred to blood culture
vials containing 10 ml of medium. Vials were immediately taken to Microbiology department. Blood culture vials were incubated at 37°C for 24 hours. Following incubation for 24 hours any coagulum or turbidity formed was noticed and if present, it was further sub-cultured and incubated on McConkey or blood agar plates. After 24 hours of incubation if any growth was seen it was noted. Identification and sensitivity of bacteria was subsequently done. Even after 24 hours if no growth was seen, culture plates was further incubated for 96 hours and again sub-cultured. After 96 hours of incubation if no growth was noted, the blood sample was labelled as sterile.

Total Leukocyte Count (TLC):

Blood was collected by heel prick method. A 1 in 20 dilution of blood was done by adding 20 ul of blood to 0.38 ml of diluting fluid (TURK'S FLUID) in WBC pipette. The suspension was mixed by rotating the pipette between palms for 1-2 minutes. The counting chamber was then charged by pipette after discarding 2-3 initial drops of suspension. By this procedure, the red cells are lysed by the diluting fluid, but the leukocyte remain intact with their nuclei stained deep violet black.
Calculations for determining total WBC count -

\[ TLC = N \times 200 / \text{cu mm.} \]

\[ N = \text{number of leukocyte counted in each mm square.} \]

**Differential Leukocyte Count (DLC):**

A thin and uniformly prepared peripheral blood smear was stained by Leishman stain for 8-10 minutes, washed with buffer water and dried in air. Leukocytes were counted using oil immersion lens and the percent distributions of different leukocytes was obtained based on counting 100 cells.

The differential count was done with special emphasis on 'Band cells' which were identified as granulocytes having non-segmented nucleus (sausage or band shaped) of chromatin, and having abundant cytoplasm (nucleus to cytoplasmic ratio 1:2).

The ratio between Band cells and total neutrophils was calculated.

**Micro ESR (mESR):**

mESR was done as described by Landau et al in 1933 and recently redescribed by Parida et al in 1980. For this blood was collected by heel prick method.
Standardised micro haematocrit tubes of 75 mm length with internal diameter of 1.1 and 1.2 mm were used. Tubes were filled completely and rapidly and one end of the tubes was sealed with plasticin. Then tubes were fixed vertically by means of sticking plasters. After one hour of fall in red cell, column was measured (distance from top of the tube to the lower miniscus of packed cell column). Values were expressed in mm.

Following cut-off values were used for sub-grouping the cases (Mishra et al, 1989).

A. Leukopenia = TLC ≤ 5000 cells/cu mm.
B. Leukocytosis = TLC ≥ 10,000 cells/cu mm.
C. mESR of ≥ 78 mm in 1st hour as raised value.
D. Abnormal Band/Total neutrophil ratio ≥ 0.2.

Statistical method:

Efficacy of a diagnostic test refers to its ability to indicate the presence or absence of disease.

Sensitivity of a test refers to its ability to diagnose the disease when it is present.

Specificity refers to the ability of the test to exclude the disease when it is not present.
Definitions:

(i) True positive cases: Confirmed cases of septicaemia by blood culture having the test positive.

(ii) False positive cases: Healthy control having the test positive.

(iii) True Negative cases: Control healthy neonates with the test negative.

(iv) False Negative cases: Confirmed cases of septicaemia by blood culture, having the negative test.

The statistical values were calculated as follows:

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\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}}
\]

\[
\text{Specificity} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Positive}}
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\text{Positive Predictive Accuracy} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}}
\]

\[
\text{Negative Predictive Accuracy} = \frac{\text{True Negative}}{\text{True Negative} + \text{False Negative}}
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