Material & Methods
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The present study was conducted in the Neonatal Intensive care unit of the Department of Pediatrics in collaboration with the department of Microbiology and Department of Pathology, M.L.B. Medical College, Jhansi. This N.I.C.U. caters to neonates delivered at home and brought directly or delivered in the college itself or referred for neonatal care from elsewhere.

CRITERIA FOR SELECTION OF CASES

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 was also recorded.

Gestational age was assessed by Ballard’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 present 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 evidence of superficial infections viz. - conjunctivitis, furunculosis, umbilical sepsis, 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

CVS, respiratory system, examination of abdomen and complete neurological examination were done in all the newborn babies. Special emphasis was given to the character of first and second heart sounds, any murmur; or signs of congestive heart failure, enlargement of liver, spleen, kidneys or any lump in abdomen. Signs of respiratory distress viz. Respiratory rate of more than 60/minute in quiet respiration, percussion and auscultation of the chest were done to exclude any respiratory problem.

A detailed neurological examination was performed and effort was made to elicit important neonatal reflexes to assess the neurological status of the newborn.
All neonates presenting with respiratory symptoms characterized by any of the following were included in the study: (i). rapid, noisy or difficult breathing; (ii). respiratory rate > 60/min; (iii). chest retraction; (iv). cough; and (v). grunting. Surgical problems causing respiratory distress, i.e. congenital malformations affecting respiratory tract and congenital heart disease were excluded from the study.

The baby was evaluated between feeds and in quiet state. Respiratory rate was recorded for at least 1 minute. The diagnosis of respiratory problems was based on guidelines recommended by the NATIONAL NEONATOLOGY FORUM (NNF).

Pneumonia was diagnosed in the presence of respiratory distress with: (a) positive blood culture or (b) if any two of the following were present (i). existing or predisposing factors characterized by any one of the following: (a) prolonged rupture of membranes ( > 24 hrs.); (ii). clinical picture of sepsis characterized by any of the following : (a) poor feeding, (b) lethargy, (c) poor reflexes, (d) hypo or hyperthermia, (e) abdominal distention; and (iii). x-ray picture suggestive of pneumonia characterized by any of the following; nodular or coarse patchy infiltrates, diffuse haziness or granularity, air bronchogram and lobar or sublobar consolidation. Transient episodes of consolidation lasting less than 48 hours due to pulmonary edema were excluded from the diagnosis of pneumonia; (iv) Positive sepsis screen.
Transient tachypnea of the newborn was diagnosed as respiratory distress in a term or borderline term neonate starting within 4 hours after birth, often requiring supplemental oxygen but recovering spontaneously within 3-4 days and showing characteristic X-ray changes, i.e. linear streaking at hilum and interlobar fluid.

Hyaline membrane disease (HMD) was diagnosed when the following three criteria are present: (a) preterm neonates; (b) respiratory distress having onset within 2 hours of birth; and (c) skiagram of chest showing poor expansion with air bronchogram or reticulogranular pattern or ground glass opacity.

Meconium aspiration syndrome was diagnosed in the presence of at least two of the following (1) meconium staining of the liquor or staining of nails or umbilical cord or skin; (2) respiratory distress soon after birth; and (3) radiological evidence of aspiration pneumonitis (atelectasis or hyperinflation).

Following investigations were done in the neonate at the time of inclusion into the study and samples, were sent to the Department of Pathology, M.L.B. Medical College, Jhansi. Sepsis screen was considered as positive if at least two of these were positive: (i) peripheral smear with bandemia more than 20%; (ii) total leukocyte count interpreted as per reference value; The total leukocyte count is usually believed to have a low predictive value for the diagnosis of
sepsis because of the wide range of normal counts from 8000 to 20,000/ cm³. Leucopenia (< 5000 /cm³) or absolute neutropenia (< 1500 /cm³) is usually associated with neonatal sepsis. A band neutrophil is an immature neutrophil, wherein the width of the narrowest segment of its nucleus is more than one third of the broadest segment.

(iii). **Micro-ESR**- interpreted as per criteria suggested earlier: and normal value is up to 6 mm in the first hour during the first 3 days of life. By the end of first month, maximum fall may be up to 11 mm during the neonatal period. A value of more than 13 mm was considered as suggestive of infection. Micro-ESR was obtained by collecting capillary blood in a standard pre-heparinized micro-hematocrit tube (75 mm length, internal diameter of 1.1 mm and outer diameter 1.5 mm) and reading the fall of erythrocyte column after one hour.

(iv) **CRP** was done by rapid slide latex agglutination method using commercial kits. These kits were available in Jhansi and a kit of 10 tests cost Rs. 350/-.

A level of more than 6 mg / L was considered as abnormal in the neonate.
Principle of the test

Rhelax CRP slide test for detection of CRP is based on the principle of agglutination. The test specimen (serum) is mixed with Rhelax CRP latex reagent and allowed to react. If CRP concentration is greater than 0.6 mg/dl a. visible agglutination is observed. If CRP concentration is less than 0.6 mg/dl, then no agglutination is observed.

Required Testing Material

1. Rhelax CRP reagent: A uniform suspension of polystyrene latex particles coated with Anti-CRP antibodies (Monoclonal IgG).

2. Positive control, reactive with Rhelax CRP reagent.

3. Negative control, non-reactive with Rhelax CRP reagent. The Rhelax CRP reagent is standardized to detect CRP concentrations greater than 0.6 mg/dl.

4. Glass slide with six reaction circles

5. Sample dispensing pipettes, Mixing sticks, Rubber teat.

6. Stop watch, Test tube, a high intensity direct light source, isotonic saline.

Specimen Collection and Storage

The samples were withdrawn within few hours of admission and serum separated after centrifugation simultaneously in the ward itself.
Fresh sera/ Plasma was used for the test. Usual precautions for venepuncture were observed. No sample pretreatment was necessary, so none were taken.

The reagent was stored at 2 - 8°C and not frozen.

1. Markedly lipemic, hemolysed and contaminated serum samples could produce non-specific results.

2. Use of plasma rather than serum can lead to false positive results.

3. Do not read results beyond indicated testing time limits.

Test Procedure

Both reagents and samples were brought to room temperature before use.

Semi quantitative method

1. Using isotonic saline prepare serial dilutions of the serum sample 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and so on.

2. Pipette each dilution of the serum sample onto separate reaction circles.

3. Add one drop of Rhelax CRP latex reagent to the drop of test specimen on the slider. Do not let the dropper tip touch the liquid on the slide.

4. Using a mixing stick, mix the sample and the latex reagent uniformly over the entire circle.
5. A stopwatch was started immediately and the slide was rocked gently, back and forth, observing for agglutination macroscopically at two minutes.

INTERPRETATION OF RESULTS

Semi quantitative method

Agglutination in the highest serum dilution corresponded to the approximate amount of CRP in mg/dl present in the specimen. Concentration of CRP was calculated as follows:

\[ \text{CRP (mg / dl)} = 0.6 \times D \]

Where D = highest dilution of serum showing agglutination.

All bacterial isolates were identified by conventional methods, X-ray chest was interpreted as per suggested criteria. Blood from radial or posterior tibial artery was taken by complete aseptic precaution in a 1ml syringe after heparinizing by a sterile concentrated heparin solution. Arterial line was used whenever available. Care was taken to ensure that the baby is not crying.

The samples for blood culture were isolated by standard methods and were sent to the Department of Microbiology M.L.B. Medical College, Jhansi. Samples, for testing the drug sensitivity of the prevalent bacteria, isolated from blood culture positive cases was done by KIRBY BAUER Method.