MATERIAL AND METHODS
MATERIAL AND METHODS

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 between August '94 to July '95 were included in this study.

Study group:

The study group consisted of 55 newborn babies delivered by normal vaginal and caesarean section. The newborn babies were classified according to their gestational age and birth weight criteria laid down by Luchenclo LO et al. (1966). On the basis of gestational age, babies were divided into 3 sub-groups - Preterm babies (≤ 37 weeks), term babies (37-41 weeks) and post-term babies (> 41 weeks). According to birth weight, babies were further classified ascertaining their position on intra-uterine growth curve (Luchenclo L0 et al., 1966).

1. Pre-term (gestational age less than 37 weeks) -

   (a) Small for gestational age (SGA), (Birth weight below 2 S.D.)

   (b) Appropriate for gestational age (AGA),
       (Birth weight upto 2 S.D.)
(c) Large for gestational age (Birth weight more than 2 S.D.).

2. Term Babies (Gestational age 37-41 weeks) -
   (a) Small for gestational age (Birth weight less than 2 S.D.)
   (b) Appropriate for gestational age (AGA), (Birth weight upto 2 S.D.).

3. Post-term Babies (gestational age 42 weeks or more) -
   (a) SGA (Birth weight below 2 S.D.),
   (b) AGA (Birth weight upto 2 S.D.).

The definition for the low birth weight babies as laid down by World Health Organization (WHO, 1961) was adopted in the present study to classify the babies with birth weight less than 2500 gms in low birth weight (LBW) group.

4. Stressed Newborn :- Baby delivered by lower segment caesarean section, Mother having pregnancy induced hypertension Antepartum haemorrhage or prolong labour (more than 15 hours).

HISTORY :

This include obstetrical history, socio-economic history, and past history. In obstetrical history, parity, abortion, previous premature birth, still birth, neonatal
death, previous LSCS, prolong labour, PEF, hydromnios, twin pregnancy were recorded in each case. Application of forceps at the time of previous deliveries was also recorded.

Emphasis was given in each case to record the history of last menstrual period and was recorded when the mother was sure of it. Gestational age was calculated in complete weeks from first day of last menstrual period and by the physical and neurological criteria laid down by Dubowitz et al (1970).

Antenatal, Natal and Post-natal History :

A detailed history of any medical or surgical disorder viz. anemia, convulsions, edema, hypertension, cardiac disorder, antepartum hemorrhage, exanthematous fever, syphilis, gonorrhoea was recorded. History of drug intake and addition to narcotics, smoking etc. were also taken in each case. Multiple pregnancies were also considered in the history.

History was taken regarding the mode of delivery, duration of labour, leaking P/V, meconium staining of liquor cry and activity of baby after birth, and cyanosis after birth to rule out any evidence of perinatal stress.

All newborn in this study were examined in detail with regard to Apgar score, birth weight, cry, activity, any congenital malformation, mode of delivery, duration of labour,
leaking P/V, muconuem staining of liquor amnii, cyanosis after birth.

Apgar scoring of baby was done at 1 minute and 5 minutes to detect any evidence of birth asphyxia.

Thorough clinical examination was done in each case. Head of newborn baby was examined in detail for the size of fontanelle, over-riding of skull bones, moulding, presence of caput succedænum, cephalhaemotoma, shape of head and any mark of injury over head. Eyes were examined for any evidence of conjunctivitis or cataract. Detailed examination was done to find out any congenital anomalies. A thorough systemic examination of cardiovascular system, respiratory system, nervous system and abdomen was also done in each case.

Anthropometric measurements viz. head circumference, chest circumference, length were recorded. Birth weight of each case was recorded within 1 hour of delivery. Neonatal reflexes viz. feeding reflexes (Rooting, sucking and swallowing). Extensor reflexes (Moro's, tonic neck reflex, galants reflexes, Perez reflexes) progeession reflexes (stepping, placing reflex) were examined in each case. 

Assessment of gestational age was done by using the physica and neurological characteristics laid down by Dubowitz et a (1970). Ten neurological characteristics were scored from 0-5, while eleven physical characteristics were scored from 0-4 in a predesigned proforma and conversion of score into
gestational age was done by the following formula or by the conversion curve (Dubowitz et al, 1970).

Collection of Sample :-

Blood sample (5 ml.) was collected from the cut end of umbilical cord from the placental site in a clean, sterilized vial with due precaution to avoid contamination with maternal blood and haemolysis. All the vials used in the study were thoroughly sterilized. Blood samples were allowed to clot at room temperature after 2-4 hrs, serum was separated using a pipette and then serum was centrifuged at 3000 - 4000 rpm for 5-10 minutes. After centrifugation 2 ml of clear serum at the top of sample was transferred to another dried vial with due marking on it. The samples were stored at +4°C and were analysed within 6-7 days for cholesterol and triglyceride.

METHOD USED FOR ESTIMATION :

Cholesterol :- Cord blood cholesterol was done by one step kit method of Wybenga and Filiggi supplied by Steranzen Immunodiagnostic using photocolorimeter.

Principle :- Cholesterol reacts with Ferric Perchlorate in presence of ethyl acetate and sulfuric acid when heated in boiling water bath to produce a lavender colour complex. The intensity of colour produced is proportional to the cholesterol concentration.
Reagent required:

1. Cholesterol reagent 250 ml,
2. Precipitating reagent 5 ml,
3. Standard (200 mg%) 3 ml.

Procedure:— Pipette into clean, dry test tubes labelled
Blanks (B), Standard (S) and Test (T).

<table>
<thead>
<tr>
<th></th>
<th>(B)</th>
<th>(S)</th>
<th>(T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Reagent (1)</td>
<td>5 ml</td>
<td>5 ml</td>
<td>5 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0.025 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (3)</td>
<td>-</td>
<td>0.025 ml</td>
<td>-</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>0.025 ml</td>
</tr>
</tbody>
</table>

Mixed well and keep the tubes immediately in the
boiling water bath exactly for 90 seconds. Cool them
immediately to R.T. under running tap water. Measure the
OD of standard (S) and test (T) against blank (B) on a
colorimeter or on a spectrophotometer at 560 nm.

Calculation:

Total cholesterol in mg% = \frac{\text{OD Test}}{\text{OD Standard}} x 200

Triglyceride:— Cord blood triglyceride was estimated by
 enzymatic kit GPO/POD method, supplied by Stanzen Immuno-
diagnostic with photocalorimeter. This test based on method
developed by Fossati and Prencipe with improved accuracy and stability. The advantages are the use of rapid simple one step enzymatic method.

**Principle:**

Triglycerides from serum hydrolysed by lipase and the glycerol that is liberated is reacts enzymatically to give a highly colored quinoneimine dye which has an absorbance maximum 545 nm. The intensity of the colour produced is directly proportional to the concentration of triglyceride in sample.

- Triglycerides $\xrightarrow{\text{lipase}}$ Glycerol + Fatty Acids

- Glycerol + AMP $\xrightarrow{\text{kinase}}$ Glycerol-1-P $\xrightarrow{}$ Phosphate + ADP.

- Glycerol-1-Phosphate + O2 $\xrightarrow{\text{ECO}}$ CO$_2$ + H$_2$O

- H$_2$O$_2$ + 4 AAP + P-chlorophenol $\xrightarrow{\text{ECO}}$ Quinoneimine dye.

**Reagent Required:**

Reagent 1. Triglycerides Enzyme Reagent.

Reagent 2. Triglycerides Standard 200 mg/L.

**Reagent 1:** Dissolve the content of the vial of reagent 1 in 6 ml of deionised/distilled water by gentle swirling. Do not shake, avoid frothing.
Procedure: Pipette into clean dry test tubes labelled Blank (B), Standard (S) and Test (T).

<table>
<thead>
<tr>
<th></th>
<th>(B)</th>
<th>(S)</th>
<th>(T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Enzyme reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>.01 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>.01 ml</td>
<td>-</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>-</td>
<td>.01 ml</td>
</tr>
</tbody>
</table>

Mixed well. Incubate at 37°C for 3 minutes, then add 2 ml of distilled water/billonized water and again mixed well and measure the O.D. of Blank (B), Standard (S) and Test (T) on photocolorimeter. Final colour is stable for 1/2 hr.

Calculation:

Serum Triglycerides in mg/dl = \(\frac{\text{O.D. of (T)}}{\text{O.D. of (S)}} \times 200\)

******