MATERIAL AND METHOD

The present study entitled "Renal dysfunction detected by \( \beta_2 \) microglobulinurea in sick neonates" was carried out in the Department of Paediatrics with assistance from Department of Microbiology and Department of Biochemistry of M.L.B. Medical College. The study period extended over 1 year.

Criteria for selection of cases:-

Study was conducted on full term babies admitted in NICU. Term babies were selected to rule out any variation in excretion of \( \beta_2 \) microglobulin due to immaturity of tubular function.

Study Group:-

Full term sick neonates in our study includes following condition.

Full term babies includes child with gestational age of 38-42 weeks. Gestational age was estimated by Ballard scoring system.

Birth Ashyxia :-

Which was diagnosed on basis of apgar score of 4 or less at 5 min.
**Septicemia** :- New born were selected by using infection scoring system given by Dr. S. Kumari (M.D.), P.K. Pruthi Lady Hardinge Medical College. A score of 5 or above was considered significant.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frank meconium stain liquor</td>
<td>2</td>
</tr>
<tr>
<td>Foul smelling liquor</td>
<td>2</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td></td>
</tr>
<tr>
<td>Prolong labour &gt; 24hr</td>
<td>2</td>
</tr>
<tr>
<td>Birth weight &lt; 2Kg</td>
<td>1</td>
</tr>
<tr>
<td>Leaking membrane more than 24hr</td>
<td>1</td>
</tr>
</tbody>
</table>

**Meconium aspiration Syndrome :-**

The diagnosis of meconium aspiration syndrome was made if two of the following factor were present.

1. Meconium stained liquour or meconium staining of skin, nail or cord.

2. Respiratory distress having onset soon after birth.
3. Radiological evidence of aspiration pneumonitis with area of atelectasis and hyperinflation.

**Control Group :-**

Healthy full term babies.

**Investigations :-**

1. ELISA for detecting $\beta_2$ microglobulin in urine.
2. Blood Urea
3. Serum creatinine
4. All other relevant investigation

**Method**

- Urine sample were collected on day 1 in all and on day 3 and 7 in those having abnormal values in first sample.
- With in half hour of collection the pH of urine was adjusted between 7-8 by adding 0.1 NaOH and store at -20°C.

**Detection of $B_2$ microglobulin in Urine**

- Enzyme immunoassay was use to determine the value of $\beta_2$ microglobulin in urine.

**Principle of test**

- Specific monoclonal anti $\beta_2$ microglobulin antibodies are coated on to microtitation well. Test urine applied and
incubated with zero buffer. If β₂ microglobulin are present it will combine with antibodies on the wall. The well is then washed and then β₂ microglobulin antibodies labelled with harseredesh peroxide enzyme is added. This result in β₂ microglobulin molecule to be sandwiched between a solid phase and enzyme link antibodies. After 30 min wells are washed with distil water on addition of substrate (TMB) a colour will develop. The concentration of β₂ microglobulin is directly proportional to the intensity of colour.

**Assay Procedure**

1. A data sheet was prepared to identify the individual well.

2. 20μl of sample was dispensed and 100 μl of zero buffer into each well and will be gently mixed for 30 seconds.

3. Incubation was done for 30 min at room temperature (20⁰C - 25⁰C) then the well content was discarded and washed five times with distilled water.

4. 150 μl enzyme conjugated was added into each well, gently mixed for 10 seconds and incubated for 30 min in room temperature end then well content was discarded and washed five times with distilled water.
5. 100 μl substrate solution was added into each well, gently mixed for five second and incubated in the dark for 20 minutes at room temperature.

6. 100 μl of stop solution was added to each well, gently shaken for 30 seconds then optical density was read using a microplate reader with a 450 nm filter.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>S_A</td>
<td>C_1</td>
<td>P_2</td>
<td>S_A-P_F</td>
<td>Standard A to F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>S_A</td>
<td>C_1</td>
<td>P_2</td>
<td>P_1-P_2</td>
<td>Patient Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>S_B</td>
<td>C_2</td>
<td></td>
<td></td>
<td>C_1 - positive Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>S_B</td>
<td>C_2</td>
<td></td>
<td></td>
<td>C_2 - negative control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>S_C</td>
<td>P_1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>S_C</td>
<td>P_1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Incubation Scheme**

1) Pipet 100μl calibration, control or patient sample

   ![Incubation Diagram]

   incubate for 30 min at room temperature

   ![Incubation Diagram]

   discard the content of the wells wash 3 time.
2) Pipet 100μl enzyme conjugate

    incubate for 15 min at room temperature

    discard the content of the wells wash 3 time.

3) Pipet 100μl substrate Solution

    incubate for 15 min at room temperature

4) Add 100μl stop solution

    leave untouched for 5 min

    Read at 450 nm

**Estimation of Blood Urea**

Estimation of Blood Urea was done by DAM method

**PRINCIPLE**

Urea reacts with hot acidic Diacetylmonoxime in presence of Thiosemicarbazide and produces a rose-purple colored complex, which is measured colorimetrically.
SAMPLE

Serum or Plasma (0.01 ml is required): (Do not use anticoagulants containing Ammonium salts)
Urine: Dilute 1:20

REAGENTS (Supplied in the Kit)
Reagent 1: Urea Reagent
Reagent 2: Diacetylmonoamine (DAM)
Reagent 3: Working Urea Standard, 30 mg%

PROCEDURE

A. For Colorimeter:

<table>
<thead>
<tr>
<th></th>
<th>Blank (B)</th>
<th>Test (T)</th>
<th>Standard (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solution I</strong></td>
<td>2.5ml</td>
<td>2.5ml</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>0.01ml</td>
<td>-</td>
</tr>
<tr>
<td>Reagent 3: Working Urea Standard, 30mg%</td>
<td>-</td>
<td>-</td>
<td>0.01ml</td>
</tr>
<tr>
<td>Reagent 2: Diacetylmonoamine (DAM)</td>
<td>Mix well</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25ml</td>
<td>0.25ml</td>
<td>0.25ml</td>
</tr>
</tbody>
</table>

CALCULATIONS:

Serum/Plasma: Urea in mg/100ml, (A) O.D. of test/O.D. of Std. x30

Blood Urea Nitrogen in mg/100 ml = (A) x 0.467

Urine: Urea in g/l, (B) O.D. of test/O.D. of Std. x 30x20/100

Urea Nitrogen in g/l = (B) x 0.467
Estimation of Serum Creatinine:

This was done by using modified Jaffe's reaction.

Principal:

Creatinine reacts with alkaline picrate to produce a reddish colour (the Jaffe's reaction). The absorption of reddish colour formed is directly proportional to creatinine concentration and is measured photometrically at 500-520nm.

Reagent use:

1) Picric Acid Reagent
2) Sodium Hydroxide Reagent
3) Creatinine Standard

Sample:

Serum was preferred but heparinise plasma may also be used. Specimen were stable for at least 2 days at room temperature and 1 week when refrigerated and longer if stored frozen.
**Assay Procedure:**

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Regent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>100 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

Mix well and read initial absorbance ($A_1$) 20 second after mixing and final absorbance ($A_2$) 80 second after mixing.

**Calculation**

\[
\Delta A = A_2 - A_1
\]

Creatinine - $\Delta A$ of sample/ $\Delta A$ of standard x Concentration of standard (mg/dl).

**Assay Procedure:**

<table>
<thead>
<tr>
<th>Pipette</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Regent</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>100 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>100 µl</td>
</tr>
</tbody>
</table>
Mix well and read initial absorbance \((A_1)\) 20 second after mixing and final absorbance \((A_2)\) 80 second after mixing.

**Calculation**

\[
\Delta A = A_2 - A_1
\]

Creatinine - \(\Delta A\) of sample/\(\Delta A\) of standard x Concentration of standard (mg/dl).