MATERIAL AND METHODS
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The present study was carried out to estimate the urea level, creatinine and uric acid level in maternal blood and cord blood of normal pregnant females and those suffering from toxæmia of pregnancy and results in both have been compared.

Selection of cases:

Estimation of the urea level, serum creatinine and serum uric acid was carried out in the following group of females:

1. Healthy non-pregnant females.
2. Healthy pregnant females.
3. Pregnant females suffering from toxæmia of pregnancy.

For convenience toxæmia of pregnancy cases were divided into three groups depending upon the severity of the disease. Three parameters were considered i.e. blood pressure, oedema and proteinuria.

GROUP A: (Mild and moderate pre-eclampsia)

Those cases in which the blood pressure was detected up to 160/100 mm of Hg with detectable edema and proteinuria.
GROUP B : (Severe pre-eclampsia)

In these cases blood pressure was raised above 160/100 mm of Hg with oedema and/or albuminuria.

GROUP C : (Eclampsia)

These patients presented with varying degree of hypertension, oedema, proteinuria with convulsions.

The healthy non-pregnant females were selected among those attending the out patients department in Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi between April 1990 to March 1991, and those admitted in ward for gynaecological problems. The normal pregnant females were selected among those admitted in Maternity Ward and Labour Rooms during the same period in some hospital, in last trimester of pregnancy, preferably within fifteen days preceding the delivery. The cases of toxemia of pregnancy were those admitted in the hospital in the third trimester of pregnancy near term.

In all these cases a detailed past and present obstetric history was taken. A thorough general examination, systemic and obstetric examination was done to note the period of gestation, presenting part, its position and engagement. Fetal heart was auscultated. Blood pressure was recorded in each patient.
Birth weight of the babies born to the above mothers was recorded in grams.

Investigations :
1. Haemoglobin estimation.
2. Albumin in urine by boiling test.
3. Estimation of urea level in maternal blood and cord blood.
4. Estimation of creatinine level in maternal blood and cord blood.
5. Estimation of uric acid level in maternal blood and cord blood.

Collection of sample :
1. Blood urea - Maternal blood was collected in an oxalate vial from the antecubital vein of the selected cases. The amount of blood taken was 2 ml.
   Cord blood was taken at the time of delivery from the baby's cord in an oxalate vial measuring 2 ml.
2. Serum creatinine - About 3 ml of maternal blood as well as cord blood were taken separately in plain vials.
3. Serum uric acid - Around 3 ml of maternal blood and cord blood was taken in two separate plain vials.

Estimation of urea level in maternal and cord blood was done by Nesslerisation method as described by King and Moeller, 1939.
**Principle:**

The sample of blood is digested by urease and the urea is thus converted into ammonia. After removal of the proteins, the colour produced by the ammonia with Nessler's reagent is compared with the colour produced under similar conditions with standard urea solution treated with urease. Direct Nesslerisation should not lead to production of cloudiness in the case of protein free filtrates from the unmixeded blood.

The sulphhydryl substances, glutathione and ergothionine which produce turbidity with Nessler's reagent because of insolubility of their mercury salt, are confined to the cells and do not appear in the filtrates with laked blood. Filtrates of unmixeded blood have further advantage that no ammonia is contributed to the determination, through the action of arginase of the blood cells on the arginine contained in the commercial preparation of urease. The use of zinc hydroxide as deproteinizing reagent eliminates the small amount of turbidity producing substances contributed by most preparations of urease.

**Method:**

(a) **Test** - 0.1 ml of blood is added to a centrifuge tube containing 4.5 ml of isotonic sodium sulfate solution. 0.1 ml of urease solution is added and the tube is stoppered with a rubber lining, mixed and incubated at 37°C for 20 minutes.
0.2 ml of zinc sulfate and 0.2 ml of 0.5 N sodium hydroxide are added to precipitate the proteins. The mixture is well mixed by inversion and centrifuged. 3 ml of supernatant fluid (0.06 ml of blood) is treated with 2 ml of ammonia free distilled water, 0.05 ml i.e. a drop of iodine solution (to prevent clouding) and 1 ml of Nessler’s reagent.

(b) **Standard** - 4.5 ml of isotonic solution in a similar tube and 0.1 ml of standard solution is added.

(c) **Blank** - 4.5 ml of isotonic solution, 1 ml of Nessler’s reagent is added to each tube and readings are taken against blank using blue filter (480 mm).

**Calculation:**

\[
\text{Blood urea} = \frac{\text{Test reading} - \text{Blank reading}}{\text{Standard reading} - \text{Blank reading}} \times 100
\]

**Serum Creatinine:**

**Principle** - Creatinine is treated with picric acid in alkaline medium, gives a red colour which is measured colorimetrically. The reaction is called Jaffey’s reaction. The method used is modified version of Brod et al.

**Reagent:**

1. Sodium tungstate, 10 percent
2. Sulfuric acid \((2/3) N\)
3. Sodium hydroxide 0.10 percent
4. Saturated picric acid solution
5. Stock creatinine standard is prepared by dissolving 100 mg of pure dry creatinine in 100 ml of 0.1 N HCl.

6. Working creatinine standard - Dilute 1 ml of stock solution to 100 ml with water.

7. Alkaline picrate solution is prepared just before use - 10 ml saturated picric acid and 2 ml NaOH.

Procedure:

Test - In a centrifuge tube 1 ml serum, 4 ml of water and 0.5 ml sodium tungstate and 0.5 ml sulphuric acid is mixed. The mixture is inverted and centrifuged. 3 ml of supernatent is taken in another tube.

Standard - 3 ml of working standard creatinine.

Blank - 3 ml of water is taken.

1.5 ml alkaline picrate solution is added to each tube and contents are mixed well and left for 10 minutes. Now the absorbance is measured using green filter (520 nm) against the blank.

Calculation:

\[
\text{Serum creatinine (mg/100 ml)} = \frac{\text{Test reading}}{\text{Standard reading}} \times 6
\]

Serum uric acid:

Principle - Uric acid is treated with phosphotungstic acid in alkaline medium. Phosphotungstic acid is reduced by uric acid forming a blue coloured complex which is measured colorimetrically.
Reagents:

1. Protein precipitant - 50 ml of sodium tungstate, 50 ml of 2/3 N sulphuric acid and a drop of phosphoric acid is mixed in 800 ml of water.

2. Phosphotungstic acid

3. Sodium carbonate 10 percent

4. Stock uric acid standard

5. Working uric acid standard is prepared by diluting 1 ml of stock standard to 200 ml with water.

Procedure:

Test - In a centrifuge tube 5.4 ml of protein precipitant solution is taken and 0.6 ml of serum is added to it. It is mixed well and centrifuged. 3 ml of supernatent is taken in another tube.

Standard - 3 ml of working standard is taken in a test tube.

Blank - 3 ml of water.

0.6 ml sodium carbonate solution and 0.6 ml of phosphotungstic acid is added to each tube, mixed and placed in a water bath at 25°C for 30 minutes.

Now the absorbance is measured within 15 minutes using red filter (700 nm) against blank.

Calculation:

Serum uric acid (mg/100 ml) = \( \frac{\text{Test reading}}{\text{Standard reading}} \times 5 \)
Toxaemia of Pregnancy

Graph No. 3

Birth weight (gms)