MATERIAL & METHODS
CHAPTER - 3

MATERIAL AND METHODS

The methodology approach used for conducting the study has been described in this research work under the following subsections.

Locality of the study: Present work is going to held in Nephrology Unit M.L.B. Medical College, Jhansi in the supervision of Dr. N. S. Senger, lecturer of Nephrology, MD, DM.

SAMPLING PROCEDURES:

Interview method (oral questionnaire method) is going to adopt for collecting information from the renal stone patients, their 24 hours urine calcium excretion and their relationship to dietary pattern as comparison to normal individual in the (nephrology unit ) OPD of M.L.B, Medical College, Jhansi, are going to select as research samples.

METHODS-

It is important that the relevant components of the 24 hours urine be carefully analysed according to the principles for the quality standard.

COLLECTION PRECAUTION -

The patients has to collect a complete 24 hours urine sample:

Instructions: How to collect the sample are essential:

(i) The first portion of the urine in the morning is discarded and the time has to be noted.
(ii) Collect all urine during the followed 24 hours in the bottle.

(iii) The last voiding should be made at the same time as the collection was started on the previous day.

(iv) The Patients has to collect a complete 24 hours urine sample without wasting a single drop of urine excretion as to fulfill the requirement of 24 hours urine excretion volume.

(v) If possible, store the urine at a cool place and bring it to analysis as soon as possible after the collection has been completed.

ESTIMATION OF

OBJECT - TO ESTIMATE CALCIUM IN SERUM / URINE.

TYPES OF METHODS -

1. Simple Titration

2. Colorimeter and

3. Flame Photometry

Titration methods are simple but not as reliable as other two techniques. Colorimeter method is more suitable for majority of the laboratories. This method is the best because of cost factor and easy working.

Methodology - According to the manufacturers protocol in the kit supplied.
NORMAL REFERENCE RANGE -

Serum Calcium: 9 to 11.0 mg / 100 ml (4.5 to 6.0 mEq / L)

Urine Calcium : 10 mEq / L

<table>
<thead>
<tr>
<th>Method</th>
<th>Test</th>
<th>Standard</th>
<th>Blank</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>S</td>
<td>B</td>
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Working Reagent: 6.0 ml

<table>
<thead>
<tr>
<th>Urine</th>
<th>0.05</th>
<th>------</th>
<th>------</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>------</td>
<td>0.05</td>
<td>------</td>
</tr>
<tr>
<td>D/oo</td>
<td>------</td>
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<td>0.05</td>
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</tbody>
</table>

Mix thoroughly, keep at room temperature for 5 minutes and read at 575 (Yellow Filter).

\[ T - B \times 10 \]

Calculation:

1. Urine Calcium mg / dl = O.D Test \( \times 10 \)

2. 24 hours Urinary Calcium Excretion.

\[ \text{Urine Calcium mg/dl} \times 24 \text{ hours Urine Volume} \]

It is better to dilute urine in 1:2 dilution of 1:5 dilution and multiplying result by dilution factor x volume Reagent I & II.

Normal Urine - 24 hours Urinary Excretion of Calcium.

Calcium = 100 - 300 mg

(50 - 150)?

Avoid or discard first urine sample (morning). The day from which collection is started and collect next day 1st morning sample.

Preservatives - (i) N/10 HCL, roughly 100 ml for 1.2 - 1.5
litre of urine.

(ii) Toulene 2ml/100ml of urine.

(iii) Thymol one small crystal/100 ml of urine.

REAGENTS :-

**Calcium Reagent I** - It is prepared by mixing 40 mg of *Cresolphthalein Complexone* in 1.0 ml of concentrated HCL(A.R.Guide) followed by 2.5 gm of *8-Hydroxy Quinoline*, 100ml of *Dimethyl Sulphoxide* and final quantity is made up to 1 litre by using glass distilled water.

**Calcium Reagent II** - It is prepared by mixing 500 mg of *Potassium Cyanide* and 40 ml *Diethylamine* in 960 ml of glass distilled water.

**Calcium Standard** - 10 mg/dl (5.0 m Meq/litre). It contains 25 mg of *Calcium Carbonate* in 50% (v/v) HCL.

4. EDTA - 4.0 mg/dl.

**Stability** - Reagent I & II are stable at room temperature for 3 months. Reagent III is stable at 2 -8°C and Reagent 4 is stable at room temperature for several months.

**Working Reagent** - Stable for one day at room temperature. Mix for 10 minutes with occasional shaking to ensure complete solution of the precipitate. Cool and add 3 ml of colour reagent and mix well.

**Blank** - 1 ml of EDTA and 3 ml of colour reagent.

Measure the absorbance using the blue filter (450 nm) against water blank.
Calculation -
Serum Calcium in mg% = Absorbance of sample

Linearity -
1. Patients receiving EDTA treatment can not be assayed for calcium correctly.
2. If the calcium value exceeds 15 mg% a suitable dilution can be made with normal saline. In such case the result obtained should be multiplied with the dilution factor to obtain the correct calcium value.

ESTIMATION OF URINARY CALCIUM (HYPERCALCIURIA)

Methods -
(i) Flame Photometry Method
(ii) Colorimetric Method
(iii) Autoanalyzer Method

Colorimetric Method being simple, cheap and convenient is preferred.

Sample Collection - 24 hours Urinary Sample is needed.

Calcium precipitating reagent - Add 5 ml of ethanolamine and 2 g of tartaric acid to about 100 ml double distilled water in a beaker. Mix, add 250 mg of naphthylhydroxamic acid powder. Stir with a glass rod and dissolve by warming. Dissolve 9 g of sodium chloride in about 250 ml water, add it to the above mixture. Transfer the whole solution to a one litre volumetric flask and raise the volume to the mark with water. If a precipitate forms, filter through a Whatman filter No. 40 or 43.

EDTA Solution - Dissolve 2 g of disodium EDTA in one litre of 0.1 N sodium hydroxide solution (4 g NaOH per litre water= 0.1N)
**Colour reagent** - Dissolve 30 g of ferric nitrate \( [\text{Fe(NO}_3\text{]}_3 \cdot 9\text{H}_2\text{O}] \) in about 250 ml volumetric flask. Add 7.5 ml of concentrated nitric acid and make up the volume to the mark with water.

**Calcium Standard (5 mEq/L)** - Dissolve 125 mg of dry Calcium carbonate in 40 ml of 0.1 N-HCL in a 500 volumetric flask. Raise the volume to the mark with water. \( (0.1 \text{ N-HCL}=0.9 \text{ ml concentrated HCL made to 100 ml with water}) \).

**Preparation of working Solution** - Prepare working solution by mixing equal volume of CPC Reagent and Diluent Reagent.

**Stability** - The reagents are stable till the expiry date stated on the bottle label; when stored at 2°-8° C.

The working solution is stable for 7 days at 2°-8° C.

**Components & Concentration of Working Solution** - Resuspend precipitate & place the stopper over the tube and heat in a water bath.