MATERIAL
&
METHODS
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To accomplish detection of asymptomatic urinary abnormalities in Bundelkhand region, it was necessary to screen the population in an effective and cheap way. Hospital was a biasing factor as most population of different regions draining into the hospital was symptomatic and suffering from variety of disease that may primarily or secondarily involved the renal system.

The best way was to conduct renal disease detection camps for the mass population in the study area with the help of health clubs and health promoting missionaries and projects.

The second problem was cost and effectiveness of the method used for screening. Eventually it was realized that uroscopy (cytobiochemical examination of urine) was a cheap and fruitful examination to screen for asymptomatic renal disease load in the population.

While screening the mass, a working format was decided for each individual attending the renal camps that included Name, Age, Sex, and Blood pressure, symptoms at presentation, urine examination, blood glucose examination and fundus examination. Individuals who had detectable urinary abnormalities were called the next day for repeat and further evaluation of the abnormalities and for reaching at a clinical diagnosis as the cause of this urinary abnormality. Of this mass of population screened a significant fraction was found to have detectable urinary abnormality and many of such patients were without clinical symptoms.
Taking the study conducted by Dr. V.N. Acharya in Bombay city as prototype, this study was conducted into 2 parts. First part comprised on initial assessment of urine (macroscopic and microscopic), blood pressure, and clinical symptoms of presenting patients and the second part was a follow up of cases that were detected to have urinary abnormality, so as to confirm the persistence of the urinary abnormality detected initially by repeat urine examination and work up and reach the cause of that persistent urinary abnormality.

For each part of this study simple working proforma were laid down and each patient that attended our renal camps was assessed according to this preplanned proforma.

**WORKING PROFORMA 1 (Renal camps):**

Included the following data for all group of people attending the renal camps :-

1. **Name:**

2. **Age:**

3. **Sex:**

4. **Blood pressure:** was recorded using mercury manometer with a 12 cm broad cuff. For children below 12 years an 8 cm cuff was used. For correct recording the deflation rate was usually kept at 2mm per second.

5. **Clinical symptoms:** Any symptoms complained by the patient that appeared to be urological, were recorded. Patients with symptoms other than renal were considered to be asymptomatic.
6. Fresh Urine examination (Routine and Microcopy):

COLLECTION:

There are 3 ways to obtain a urine specimen – spontaneous voiding, urethral catheterization, and suprapubic bladder puncture. Spontaneous voiding is the simplest and best method, if specimen is collected appropriately. A clean catch urine sample was obtained. In males, foreskin was retracted and glans penis cleansed. Similarly, in females, the labia were separated and area of labia and urethral meatus cleansed. Then midstream urine was collected. The present study used the spontaneous voiding method.

MACROSCOPIC EXAMINATION:

Urine sugar:-

In this study urine sugar was tested by benedict’s method

BENEDICT’S TEST: - This test is based on the ability of sugar in urine to reduce cupric ions to cuprous ions. Though the test is not specific but it is better and more specific than the fehling’s solution test. Benedict’s qualitative reagent – was prepared by dissolving 173 g of sodium citrate and 100 g of anhydrous sodium carbonate in about 600 ml warm water. This was filtered into a one liter volumetric flask. Then 17.3 g of copper sulphate (CuSO4. 5H2O) in about 100 ml water in a beaker and added slowly to the above mixture with constant shaking. This final mixture was made 1 litre by adding water. Procedure: - 5 ml of Benedict’s reagent was taken in tube and to it 0.4 ml (8 drops) of urine was added. The tube was heated directly on the flame till
the solution boiled. This was then cooled and examined for change of color.

<table>
<thead>
<tr>
<th>Observation</th>
<th>Clinical record</th>
<th>Approx. glucose concentration (gram/100 ml urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>0</td>
<td>Nil</td>
</tr>
<tr>
<td>Green (no precipitate)</td>
<td>+</td>
<td>Traces</td>
</tr>
<tr>
<td>Green (with precipitate)</td>
<td>+</td>
<td>0.5</td>
</tr>
<tr>
<td>Brown</td>
<td>++</td>
<td>1.0</td>
</tr>
<tr>
<td>Orange</td>
<td>+++</td>
<td>1.5</td>
</tr>
<tr>
<td>Red</td>
<td>++++</td>
<td>2.0 and over</td>
</tr>
</tbody>
</table>

Note: - In addition to glucose the reduction of Benedict's solution may be caused by the following (false positive): (i) other sugars including lactate, fructose and pentoses. Lactose is the commonest, particularly during late pregnancy or lactation. (ii) Normal urine constituents, particularly uric acid, creatinine and ascorbic acid. Reduction is slight and only occurs with concentrated urines. (iii) The end products of drugs, commonly aspirin and salicylate (which are excreted as glucuronides and salicyluric acid).

**Urine protein:** – The presence of protein in urine is called proteinuria. Albumin is the main constituent, though higher molecular weight globulins also appear in the urine. Various simple methods for testing urine protein are available.
HEAT PRECIPITATION TEST- In this test the adjustment of urine pH to about 5 is necessary because the proteins only coagulate when they are heated at a pH near their isoelectric point (pH 5). False negative results may otherwise be obtained if the urine is alkaline or more acidic. Furthermore, by adjusting the pH before heating, the difficulties caused by the precipitation of phosphates can be avoided.
Reagents used :-
Acetic acid 33% solution.
Procedure – Take a test tube almost full of clear urine and test the pH with litmus paper or narrow range pH paper and adjust by drop wise addition of 33 percent acetic acid until it is slightly acidic (about pH5) . Heat the top few centimeters of the urine column to boiling and note any turbidity by comparison with the unheated part of the liquid. Appearance of turbidity or precipitate confirms the presence of protein.

MICROSCOPIC EXAMINATION:

Preparation of urine sediment is the first step in microscopic analysis. The importance of standardization of technique and quality accuracy cannot be over stressed to ensure accurate and reproducible analysis. Important steps include centrifugation, resuspension of sediment, slide preparation, and microscopic examination. In brief 10 ml of urine was centrifuged at approximately 2,000rpm (1,000-2,500rpm) in a centrifuge machine for 5 minutes. The supernatant 9ml was discarded and sediment was resuspended in 1ml. A drop of this was pipette onto a slide and a cover slip placed. The slide was then examined without staining. The slide was examined both under light and high power field in the microscope the following:

a). Pus cells : A value of more than 6 pus cells / HPF was significant in this study.
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a). Pus cells: A value of more than 6 pus cells / HPF was significant in this study.
b). **RBCs**: A value of more than 5 RBCs / HPF was considered significant in this study.

c). **Crystals**: presence of any number or variety of crystals was considered as urinary abnormality in this study

### 6. Blood glucose:

Depending upon clinical symptoms and urine examination results, a blood glucose examination was done where needed. Conditions like, presence of sugar or protein, or pus cells in urine, presence of hypertensive and symptom like obesity, polyuria, nocturnal, polydipsia all demanded a blood glucose examination and so it was conducted in all such cases.

### 7. Fundoscopy:  
Fundus was examined in all cases having diabetes mellitus or hypertension to check diabetic retinopathy or vessel changes suggestive of hypertensive.

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**WORKING PROFORMA 2 (follow up):**

This included at our hospital the following examination and information.

1. **Name:**

2. **Age:**
3. Sex:

4. Repeat Blood pressure:

5. Clinical Symptoms: Presence of clinical symptoms suggestive of renal disease were asked for and signs for renal disease looked for.

6. Repeat urine(routine/microscopy): Same method was used as used in the working proforma 1.

7. Serum creatinine:
   
   Principle: Creatinine is treated with picric acid in alkaline medium, a red colour develops which is measured colorimetrically. The reaction is not specific but at least over 85 percent colour is due to creatinine
   
   Reagents used:-
   
   -Sodium tungstate 10 %
   -Sulphuric acid (2/3 N)
   -Sodium hydroxide, 10 percent
   -Saturated picric acid solution
   
   -Stock creatinine standard – Dissolve 100mg of pure dry creatinine in 100mg of 0.1 N-HCl
   -Working creatinine standard-Dilute 1.0 ml stock solution to 10 ml with water.
   
   -Alkaline picrate solution- Prepare just before use a mixture of 10 ml saturated picric acid and 2 ml sodium hydroxide.

   Procedure:-
Test – in a centrifuge tube 1.0 ml serum was taken. 4 ml water and 0.5 ml sodium tungstate and 0.5 ml sulphuric acid was added to the serum. Solution was mixed by inversion and centrifuged after some time. 3 ml supernatant was taken in another tube.

Standard – 3 ml working standard
Blank – 3 ml water.

1.5 ml alkaline picrate solution was added to each tube. Mixed well and allowed to stand for 10 minutes. The absorbance using green filter (520 nm) against the blank was measured. Calculation:

\[
\text{Serum creatinine} = \frac{T \times 6 \text{ (mg/100ml)}}{S}
\]

The normal range of serum creatinine is 0.1 to 1.2 mg/100ml.

Increased values are usually found in advanced cases of renal disease. With severe renal failure it may rise to over 10 mg/100ml.

8. Repeat Blood Glucose (Fasting): Repeat blood glucose was done. This value was fasting as all cases called for follow up were told to stay 10 hour empty stomach before coming for follow up.

9. Urine Culture: Was done in all patients found to have pus cells in their urine. A clean catch sample was collected in a test tube and a loopful was inoculated on 2 types of media for 24 hours. The 2 media used were MacConkey’s agar and Blood agar. Growth was observed for and interpreted as follows: More than 10^5 organisms per ml indicated definite infection from that species, between 10^2 and 10^5 organisms indicated possible infection and less than 10^2 organisms from a single strain excluded infection.
10. Renal biopsy: Biopsy was not done in all patients found to have urinary abnormalities. It was only done in cases where the diagnosis was not clear. It was done in the morning after an overnight fasting. An i.v. access was established and maintained for the next 24 hours. Prior to the biopsy the biopsy sight was localized by USG. The Patient was made to lie in a proned position and skin cleaned, draped and infiltrated with local anesthetic agent. A seven inches needle (mostly lumbar puncture needle) was used to explore the kidney (movement of the needle was looked for during respiration). The biopsy needle used by in this study was a true cut needle (11.4cm). After adjusting the length of the true cut needle the biopsy was done. Adequacy of the sample was checked. If inadequate sample, the procedure was repeated. After taking adequate sample, it was transferred into a formalin (10 %) solution in a test tube and send for histopathology and immunoflourescent study. Results were usually obtained within 2-3 days. Care was taken in regards to the patient for the next 24 hours and features like post biopsy hematuria and hypertension and hypotension were specially monitored for in these 24 hours.

11. Final diagnosis: Using above mentioned methods and procedures cases detected to have asymptomatic urinary abnormalities were diagnosed of their underlying disease.