MATERIAL

AND

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The present study was conducted in the department of Pediatrics, M.L.B. Medical College, Hospital, Jhansi, over a period of two years from August, 1991 to July, 1993. The study was primarily aimed to study the clinical as well as immunological profile of nephrotic syndrome in Bundelkhand region.

Accordingly on the basis of history, general and systemic clinical examination, investigations, all cases of nephrotic syndrome admitted in Pediatric ward and attending Pediatric OPD formed the basis of present study.

CLINICAL DIAGNOSIS

History

A detailed present, past and relevant history was recorded in each and every case. Typical present history in a classical case was that of generalized swelling, usually starting as puffiness of eyes, particularly noted in the morning and history of low output of urine. History of gross hematuria, headache, nausea and vomitings was asked in each case.

History of infections, particularly pyoderma, upper respiratory tract infections, malaria, drug intake was also obtained.

Age, sex, family history, history of any underlying systemic disease, allergies were recorded in each case.
Past history was elicited in each case so that full account of relapses and remissions could be known.

PHYSICAL EXAMINATION

A thorough general and systemic examination was conducted in each and every case and findings were recorded on the predesigned proforma. During general examination emphasis was given to elicit oedema whether pedal, anterior abdominal wall, scrotal, periorbital. Signs of steroid effect and blood pressure were carefully monitored in each case.

In examination of respiratory system especially evidence of hydrothorax was searched in each case. In examination of abdomen, ascites, hepatosplenomegaly, anterior wall oedema were carefully looked for. Kidneys were palpated for any renal anomaly and renal angles were examination. Cardiovascular and central nervous system was watched carefully.

Children were examined for evidence of any multi-system disorders like S.L.E., Rheumatoid arthritis, Henoch Schonlein purpura, Sjogren's syndrome, tuberculosis etc. which may provide some etiological clue to the disease process.

LAB. DIAGNOSIS

After taking a detailed history and doing a thorough examination, following investigations were performed.
Urine

a. Bed side urine examination for albumin: By heat and acetic acid test was performed regularly and also taught to the parents of the patients.

Interpretation:
- No cloudiness,
+ cloudiness barely visible (traces)
++ Definite cloudiness, no granularity/flocculation.
+++ Granular cloudiness. No flocculation (0.1% protein).
++++ Dense opaque cloud, clearly flocculated (0.2 - 0.3% protein).
+++++ A thick/ almost solid precipitate (70.5% protein).

b. Sugar: By benedicts qualitative glucose test.

c. Microscopic: Clean fresh morning mid stream sample was collected and centrifuged and examined for R.B.C's, pus cells, casts, epithelial cells.

d. Specific gravity: Specific gravity was measured by urinometer.

e. Quantitative estimation of protein in urine:
- Urine collection: Child was asked to void the bladder and urine was collected in a clean large bottle for next 24 hours.
- 24 hour urinary protein was estimated by Esbach's albuminometer.
- Massive proteinuria was defined as excretion of 40 mg or more per m² of surface area or 750 mg/kg/day.

2. **Routine Blood Examination**

   Total leucocyte count (TLC), differential leucocyte count (DLC) by Newbaur's chamber, diluting fluid used, turk's solution were done to rule out underlying systemic or acute infections. ESR by Wintrobe's method and hemoglobin estimation was also done in selected cases.

3. **Blood Urea**: It was estimated by Nessler's method.

4. **Serum Cholesterol**: It was estimated by routine method of Wybenga et al using photcolorimeter.

5. **Serum Creatinine**: It was also estimated by photcolorimeter.

6. **Serum Proteins**: Serum proteins were estimated by Reinhold method and serum albumin by Biuret's method.

7. **Radiography**: Skiagram chest PA view was done in cases suspected of having primary complex.

**DIAGNOSIS OF NEPHROTIC SYNDROME**

After conducting thorough clinical examination and investigations cases were diagnosed on basis of criteria laid down by international study of kidney diseases in children (ISKDC) viz.

a. Generalised swelling.

b. Proteinuria 740 mg/m²/hour determined qualitatively on 24 hour collected urine sample or 750 mg/kg/day.
c. Hypoalbuminemia ≤ 2.5 gm serum albumin.
d. Hypercholesterolemia ≥ 200 mg% serum cholesterol.

**HISTOPATHOLOGIC METHODS**

As most of the cases of minimal change nephrotic syndrome respond to usual steroid therapy and most of the cases not responding to steroid therapy are not MCNS type, renal biopsy was performed to only in steroid resistant or frequent relapses or steroid dependent or age more than 7 years or presenting with gross hematuria and hypertension, so that underlying pathology of the lesion could be underlined. Cases were carefully followed up to study the progress of disease following therapy before they were labelled as steroid resistant, dependent or frequent relapses. Renal tissue was obtained by percutaneous biopsy performed under local anaesthesia by trucut needle (travenol). Biopsy was fixed in formaline and tissue processed in auto-technicon. Paraffin imbedded blocks were prepared and sections were cut at 5 μ thickness by microtome. Staining was done by H & E technique. Slides were made and studied under light microscope. The changes which were especially looked were cellular proliferation, glomerular basement thickening (seen by staining with PAS e.g. in membranous glomerulopathy) and focal sclerosis of glomeruli.

**IMMUNOLOGICAL METHODS**

Quantitative determination of individual serum immunoglobulins (IgG, ...) was done by Radial Immuno diffusion by technique.
Collection of Sera

Serum was separated from blood samples and stored in sterile vials in refrigerator. Samples were drawn both in nephrotic and remission phase.

Gel Diffusion Procedure

Three wells in agar plate were filled with 3 dilutions of reference standard 100%, 50% and 25%. Remaining 9 wells of plate were filled with 9 serum samples in which estimations were to be carried out. Care was taken not to underfill or over flow the wells. It was precisely filled to the brim by using capillary tubes or by syringes with 26G needle.

Plate was left for development of precipitin ring in inverted position (only after lid of plate was replaced and kept aside for 10 minutes). Plate was left for IgM for 24 hours at room temperature and another 24 hours at 4°C and for IgG over night for 18 hours at room temperature. During this period immunoprecipitate was formed. Each individual antigen produces a single precipitin line, locations of which depends upon the rate of diffusion and concentration of antigen. Diffusion rate of protein depends upon molecular weight.

Higher molecular weight globulin are precipitated in proximity of point of application. Low molecular weight proteins are precipitated nearer to antibody well.
Immunoprecipitation rings are carefully measured and standard graph is constructed using values of reference standard (Standard curve). Semilog graph paper was used to draw graph. Diameter of rings is plotted on linear scale while the quantitative value (100%, 50% and 25%) was plotted on log scale. The values of unknown samples were found out directly by interpolation on standard graph.

Statistical Methods

Statistical significance was assessed by student 't' test.

FOLLOW UP OF CASES

Patients were put on steroids and followed up. On the basis of results and clinical examination they were grouped as follows after careful follow up.

A. Steroid responders.

B. Steroid dependent.

C. Non responders to steroids.

A. STEROID RESPONDERS

Those patients who became urine albumin free within 4 weeks of onset of steroid therapy, persisting for minimum of 2 months after termination of therapy in dosage of 60 mg/m² or 2 mg/kg/day Prednisolone. These were again divided into:
a. **Frequent Relapsers**

Those patients who had 2 relapses within 6 months or 3 relapses in a year even though they responded to prednisolone therapy.

b. **Infrequent relapsers**: Less than 3 per year or ≤2 per 6 months.

B. **STEROID DEPENDENT**

In these patients proteinuria recurred when dose was reduced below a critical level (when put on alternate day regime) or proteinuria occurring within 2 months after termination of treatment on at least 2 successive occasions.

C. **NON RESPONDERS TO STEROIDS**

a. **Early non responders**: Failure to achieve remission with a initial 28 days course of prednisolone.

b. **Late non responders**: Failure to achieve remission with 28 days course of prednisolone after one or more steroid induced remissions. Before labelling as non responders, presence of infection was carefully excluded.

According to disease activity patients were placed in following phases:

1) **Nephrotic Phase**:
   - Initial episode
   - Relapse.
ii) Remission Phase:

- Unstable remission: Remission requiring steroids for maintenance of disappearance of urinary albumin and normal serum albumin.
- Stable remission: Remission maintained despite withdrawal of steroid.

DEFINITIONS

Remission: No oedema and urine free of protein by qualitative testing for consecutive 5 days.

Relapse: Oedema or first morning sample of urine contains $\gamma$-reaction for 7 consecutive days.