MATERIAL AND METHODS

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The study has been conducted on the patients of diabetic nephropathy, attending the diabetic and nephrology clinics of out patient department and admitted in M.L.B. Medical College, Hospital, Jhansi.

The history was taken from all the patients of diabetes to know the duration of symptoms, year of diagnosis, type of diabetes, familial relation and complication of disease.

General as well as systemic examinations were recorded to know the general condition, pulse, blood pressure, temperature, pallor, icterus, cyanosis, clubbing, oedema, hydration and lymphadenopathy. Systemic examination was done to find out the changes in all systems due to diabetes. The patients having disease other than diabetes were excluded from the study.

Dipstick test and fundoscopy were done to find out the proteinuria and retinopathy. After confirmation of the diabetic nephropathy, the patients were investigated for 24 hours proteinuria, blood urea, serum creatinine and glomerular filtration rate.

The blood pressure was recorded before starting drugs. The captopril and lisinopril were given to the alternate patient respectively. The doses of drugs were adjusted according to the blood pressure response. Hypoglycemic drugs were given according to blood sugar levels.

After 8 weeks course of converting enzyme inhibitors, patients were further investigated for 24 hour urine protein excretion, blood urea, serum creatinine and GFR. At last, the results were compiled to know the efficacy of drugs in relation to reduction in proteinuria and improvement in renal functions of individual drugs as well as statistical analysis was done to find out the difference in efficacy.

URINE PROTEIN

A quantitative estimation of protein was done by turbidity method as described by Wooton (1964).

Reagent

Sulphosalicylic acid (3%) : 3.0 gm of sulphosalicylic acid was dissolved in 100 ml distilled water.

Protein standard solution (100%) : In 100 ml distilled water, 100 mg crystalline bovine albumin was dissolved and kept in refrigerator.

Procedure

Twenty four hours urine was collected in bottle which was marked in milli litres so the 24 hours urinary volume was measured and noted. Toluene was used as preservative. Required amount was then taken from it and centrifuged.

Test

1.	Urine	1.0 ml
2.	Sulphosalicylic acid solution	4.0 ml

Standard

1.	Protein standard solution	1.0 ml
2.	Sulphosalicylic acid solution	4.0 ml

Blank

1.	Urine	1.0 ml
2.	Water	4.0 ml

The tubes were kept for 30 minutes and then the absorbance was measured with blue filter (450 nm) against reagent blank.

Calculation

$$\text{Urinary protein (mg/l)} = \frac{\text{Reading of test}}{\text{Reading of standard}} \times 1000$$

BLOOD UREA (Diacetyl Monoxime Method)

Reagents

1. Diacetyl mono-oxime reagent : 2 gm pure diacetyl mono-oxime was dissolved in 60 ml double distilled water and 2 ml glacial acetic acid was mixed and made it 100 ml with double distilled water.

2. Acid mixture : 150 ml of 85% phosphoric acid was added to 140 ml of double distilled water. Mixed well 50 ml of concentrated sulphuric acid was added slowly.

3. Trichloroacetic acid solution : 10 gm TCA dissolved in 100 ml double distilled water.

4. Urea stock standard : 250 mg of pure urea crystals dissolved in 100 ml double distilled water.

5. Working urea standard : 1 ml of stock solution was diluted in 100 ml of double distilled water. This solution contains 0.25 mg/urea/ml.

METHOD

0.1 ml blood sample was added in a tube containing 1.9 ml double distilled water. It was mixed thoroughly and 2.0 ml 10% TCA solution was added and centrifuged the content at 3000 rpm for 5 to 10 minutes. 2 ml supernatant was taken in a test tube and it was marked as 'T' representing the test blood sample filtrate.

Standard

1.0 ml of working urea solution was added in 1.0 ml of double distilled water. It was marked as 'S' representing standard solution.

Blank

2.0 ml double distilled water was taken and it was marked as 'B' representing the blank system.

There after 0.4 ml diacetyl mono-oxime reagent and 1.6 ml acid mixture were added in each system. Mixed well and incubate for 30 minutes in boiling water bath. After that test tubes were taken out and measured the samples at 480 nm by setting the spectrophotometer zero with the blank system.

Calculation :

$$\text{Blood urea (mg/dl)} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 100$$

SERUM CREATININE (Alkaline picrate method)

Reagents

1. Sodium hydroxide 0.75 N solution : It was prepared by dissolving 30 gm of sodium hydroxide in 1 litre of double distilled water.
2. Picric Acid Solution : Picric acid solution was prepared in double distilled water. This solution is called to be saturated when some crystals of picric acid settled down at the bottom and did not dissolve even after thorough mixing.
3. Sodium Tungstate (57 W/V) : It was prepared by dissolving 5.0 gm pure sodium tungstate in 100 ml double distilled water.
4. Sulphuric acid (2/3 N) : It was prepared by mixing 10 ml concentrated sulphuric acid to the 900 ml double distilled water and made upto the 1 litre with water.
5. Stock Creatinine standard : 20 mg pure creatinine was dissolved in 100 ml double distilled water. It was 20 mg% creatinine solution.
6. Working creatinine standard : 1.0 ml of creatinine stock solution was diluted with 10 ml double distilled water. It was 2 mg% creatinine solution.

METHOD

Sets of 3 test tubes were taken and marked as 'T' for test, 'S' for standard and B for blank. Solutions were taken as follows :

In 'T' test tube : 1.0 ml of serum and 1.0 ml double distilled water.

In 'S' test tube : 2 ml working creatinine standard solution.

In 'B' test tube : 2.0 ml double distilled water.

After that 1 ml 5% sodium tungstate solution and 1 ml 2/3 N sulphuric acid were mixed and centrifuged for 5 to 10 minutes at 3000 rpm.

In another set of the test tubes marked with 'T' 'S' and 'B', the 2 ml supernatant was taken from the above respective systems. Now 0.5 ml picric acid solution and 0.5 ml 0.75 N sodium hydroxide solution were used respectively in each system. It was incubated at room temperature for 20 minutes and readings were taken from the test and standard samples at 520 nm by setting the spectrophotometer at zero with the blank.

Calculation

$$\text{Serum creatinine (mg/dl)} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 4$$

GLOMERULAR FILTRATION RATE (GFR)

It was calculated with the help of following formula :

$$\text{Cl Cr (ml/min)} = \frac{(140-\text{age}) \times \text{weight (kg)}}{72 \times \text{serum creatinine (mg/dl)}} \times 0.85 \text{ for women}$$
