STUDY OF MICROALBUMINURIA IN DIABETES TYPE 2 PATIENTS AS A MARKER OF MORBIDITY (A STUDY OF 100 CASES IN RAJKOT CITY)

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Abstract

Background: - Diabetes is a stage of persistent hyperglycemia due to absolute or relative deficiency of insulin. About 20-40% of patients with type 2 diabetes develop evidence of nephropathy. Diabetic nephropathy is first recognized as proteinuria. Microalbuminuria is defined as the range in between urinary excretion of albumin of 20-200 µg/min or 30-300 mg/24 hrs with a negative dipstick test.

Methods: - The study was carried out on 100 patients of diabetes type 2 having more than five years duration of diabetes in Pathology Department. Patient's age, age at detection, sex, duration of diabetes, levels of Fasting Blood Sugar(FBS), Postprandial Blood Sugar (PP2BS), Urea, Creatinine, Cholesterol, Glycosylated Hemoglobin(HbA1C).

Results: - We found prevalence of microalbuminuria in present study to be 50%. A high percentage of hypertensive patients, patients having high FBS, PP2BS, cholesterol developed microalbuminuria. The group of patients having high urea and high creatinine has higher prevalence of microalbuminuria. Higher prevalence of microalbuminuria was seen in patients having high HbA1C.

Conclusion: - As it is possible to halt or delay the progression of diabetic nephropathy by the use of appropriate treatment modalities such as angiotensin converting enzyme inhibitors at this stage, the study is helpful thereby in reducing morbidity and increasing life expectancy of patients with diabetes mellitus type 2.

Keywords: Microalbuminuria, diabetes mellitus, urine albumin, diabetic nephropathy

1. Introduction:

Diabetes is a stage of persistent hyperglycemia due to absolute or relative deficiency of insulin. The disease is characterized by metabolic abnormalities and by long term complications involving eyes, nerves, blood vessels and kidneys. During the pre-insulin era the mean life expectancy from the time of diagnosis was only 5 years, with advent of insulin therapy; the mean life expectancy has progressively increased and is now more than 20 years. With this increase in longevity the frequency of deaths from renal disease has also risen. Involvement of kidney in diabetes is the leading cause of renal failure 1.

Diabetes is a global problem. The problem statement of diabetes and its complications globally accounts 6.6%, in India 9% in urban population and in Gujarat it is around 10%.Diabetes is one of the most common non communicable diseases found in our country as well as globally because of urbanization. Another 2.5% of diabetics are estimated to have the disease without knowing its existence to them 2. Diabetes has become the single most common cause of end stage renal disease. About 20-40% of patients with type2 diabetes develop evidence of nephropathy. Diabetic nephropathy is first recognized as proteinuria. The main reason for performing the test for proteinuria is for the early detection of diabetic nephropathy in a patient who had diabetes for several years. Normally, protein is not present in the urine when measured by routine Dipstick Quantitative Test. This is because glomerulus generally prevents large molecules from entering renal filtrate. Normally less than 150 mg of proteins per day are excreted in urine. About 1/3RD of protein is comprised of urine albumin, 1/3RD of small globulins, and 1/3RD of TammHorsfall Protein. Most of the proteins are normally reabsorbed by the proximal tubular epithelial cells.

PROTEINURIA is referred to dipstick positive or Albumin Excretion Rate (AER) more than 200 µg/min or 300 mg/24 hrs. 3. Microalbuminuria is defined as the range in between urinary excretion of albumin of 20-200 µg/min or 30-300 mg/24 hrs. With a negative dipstick test 4. The Microalbuminuria is also defined as urinary albumin to creatinine ratio. A ratio of greater than 30-300 mg/gm. of creatinine is considered as Microalbuminuria 4.

The central abnormality in Diabetic nephropathy is renal extracellular matrix accumulation in the mesengium 5. Diabetic kidney disease is one of the most frequent causes of End Stage Renal Disease (ESRD).
Table 1 Measurement and significance of urine albumin excretion:

| URINE SAMPLE                  | Significance |
|-------------------------------|--------------|
| **Spot collection** | **24 hr. collection** | **Timed collection** |
| µg/mg creatinine   | mg/24 hr.    | µg/min    |
| <30                  | <30         | <20         | Normal         |
| 30-299              | 30-299      | 20-199      | Microalbuminuria |
| ≥300                | ≥300        | ≥200        | Macroalbuminuria |

![Image of a graph showing time from onset of diabetes in years against GFR, M/C, and gross proteinuria.]

1.1 Aims and Objectives: (1) To detect the prevalence of microalbuminuria in diabetic type 2 patients. (2) To correlate microalbuminuria with age and sex of patients. (3) To correlate microalbuminuria with age of onset and duration of diabetes. (4) To correlate microalbuminuria with Blood sugars [Fasting Blood Sugar (FBS), Postprandial Blood Sugar (PP2BS) and Glycosylated Hemoglobin (HbA1C)]. (5) To correlate microalbuminuria with the complications of diabetes like diabetic nephropathy (Blood Urea and Serum Creatinine), hypertension (Blood Pressure) and dyslipoproteinemia (Serum Cholesterol).

2. Materials and methods:
The urine samples, which were negative for multi reagent urine analysis strips were checked for microalbuminuria by latex turbidimetry method. (Test kit manufactured by SPINREACT, S.A.) This test is a quantitative determination of micro albumin.

2.1 Principle: Latex particles coated with specific human anti-human albumin are agglutinated when mixed with sample containing micro albumin. The agglutination causes an absorbance change, dependent upon the micro albumin contents of the patient sample that can be quantified by comparison from a calibrator of known micro albumin concentration.

2.2 Reagents: Table 2

| Diluents (R1) | Glycine buffer 100 mmol/L, pH 10.0. Sodium azide 0.95 gm/L |
|---------------|----------------------------------------------------------|
| Latex(R2)     | Suspension of latex particles coated with anti-human albumin, pH 8.2. Sodium azide 0.95 gm/L |
| Calibrator (R3) | Human origin |

2.3 Preparation:-
**Working reagent:** Shake the latex vial gently before use. 1 ml latex reagent (R2) + 9 ml Diluents (R1)

2.4 Samples: Fresh urine sample, centrifuged and pH adjusted at 7.0 with NAOH/HCL 1 mol/L.

**Procedure:-**
1. Bring the working reagent and photometer to 37º C. Wavelength: 540 nm (530-550) Temperature: 37º C.
2. Pipette into a cuvette:

| Table 3                  | Calibrator | Sample |
|--------------------------|------------|--------|
| Working Reagent (ml)     | 1.0        | 1.0    |
| Calibrator (µl)          | 7          | -      |
| Sample (µl)              | -          | 7      |

3. Mix and read the absorbance immediately (A1) and after 2 minutes (A2) of sample addition.

2.5 Calculation:-
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\frac{(A2 - A1)_{sample}}{(A2 - A1)_{calibrator}} x \text{calibrator concentration} = \frac{mg}{L} \text{microalbumin}
\]

3. Discussion and results:
The present study was carried out on 100 patients of diabetes type 2 having more than five years duration of disease. Patient’s age, age at detection, sex, duration of diabetes was used and negative samples with dipstick were measured by “turbidimetry method”. Patient’s blood pressure, blood glucose, serum creatinine, blood urea, serum cholesterol and glycosylated hemoglobin were estimated. Findings were tabulated and compared with study of other workers.
The prevalence of microalbuminuria in diabetic patients in the present study was 50%.

The age of diabetic patients selected for study was varied from 40 to 75 years, with a mean age of 57.5 years. There was no significant correlation found between age and microalbuminuria as compared to normoalbuminuria.

There was no significant correlation found between microalbuminuria and sex, age at onset of diabetes, or duration of diabetes.

Correlation of microalbuminuria with blood pressure:

### Table 4

| Study                      | Normotensive | Hypertensive | No. of patients with microalbuminuria |
|----------------------------|--------------|--------------|---------------------------------------|
| Vishwanathan et al. (1991) | 28%          | 28.7%        | 90/316                                |
| Giri (2000)                | 20%          | 31.9%        | 47/82                                 |
| Parikh (2001)              | 20%          | 27.5%        | 23/100                                |
| Present study (2012)       | 41.7%        | 58.3%        | 50/100                                |

A high percentage of hypertensive patients (58.3%) had microalbuminuria and significant correlation found between microalbuminuria and blood pressure.

Correlation of microalbuminuria with FBS and PP2BS:

### Table 5

| FBS     | Number of patients |                  |                        | Percentage (%) of patients with microalbuminuria out of 100% patients |
|---------|--------------------|------------------|------------------------|---------------------------------------------------------------|
| >120 mg/dl  | Total              | With normoalbuminuria | With microalbuminuria |                                                                 |
| 50       | 26                 | 24               |                        | 48%                                                            |
| >120 mg/dl| 50                 | 20               | 30                     | 60%                                                            |
| Total    | 100                | 46               | 54                     |                                                                |

A high percentage of diabetic patients (60.0%) having FBS > 120 mg/dl had microalbuminuria and 80.6% of diabetic patients having PP2BS > 250 mg/dl had microalbuminuria. So significant correlation found which was comparable with studies done by Park et al. (1960), Hashim et al. (2003).

Correlation of microalbuminuria with serum creatinine:

### Table 6

| Study                      | S.Creatinine (mg/dl) | No. of patients with microalbuminuria |
|----------------------------|----------------------|---------------------------------------|
|                            | 0.6-1.2              | >1.2                                  |
| Parikh et al. (2001)       | 26.4                 | 0                                     | 23/100                                                             |
| Present study              | 40.5                 | 60.1                                  | 60/100                                                             |

A group of patients having serum creatinine more than 1.2 mg/dl had maximum percentage (60.1%) of patients with microalbuminuria. So significant correlation found between microalbuminuria and serum creatinine which was comparable to other study done by Farkas et al.
• Blood Urea was also found significantly higher in patients with microalbuminuria than normoalbuminuria.
• Correlation of microalbuminuria with serum cholesterol:

| Study       | S. cholesterol (mg/dl) | No. of patients with microalbuminuria |
|-------------|------------------------|---------------------------------------|
|             | <220                   | >220                                  |
| Giri² (2000)| 5.71                   | 21.28                                 | 47/82                                 |
| Parikh³ (2001)| 22.51                  | 25                                    | 23/100                                |
| Present Study (2012) | 49                     | 58.87                                 | 50/100                                |

As cholesterol level increases, the percentage of patients having microalbuminuria also increases. (49.00% with S.cholesterol< 220 mg/dl and 58.57% with S.cholesterol> 220 mg/dl). So significant correlation was found between microalbuminuria and serum cholesterol levels.

• We defined HbA1C ≤ 7 % as controlled diabetes mellitus whereas those having HbA1C ≥ 7 % as uncontrolled diabetes mellitus. Here we found higher prevalence (90%) of microalbuminuria in uncontrolled group as compared to control ones.

Conclusion:
Elderly patients with diabetes mellitus type 2 are at greatest risk of premature death by suffering from diabetic nephropathy. This justified the use of this screening test in those patients to detect early renal injury.
It is possible to halt or delay the progression of diabetic nephropathy by the use of appropriate treatment modalities such as angiotensin converting enzyme inhibitors at this stage. Thereby reducing morbidity and increasing life expectancy of patients with diabetes mellitus type 2.

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