

## Detection of Renal Tubular Proteins: An Indication of Tubulo-interstitial Nephrosis in Diabetic patients on Insulin

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### ABSTRACT:

**Background:** Diabetic nephropathy in patients on insulin is characterized by glucose-induced inhibition of matrix-degrading enzymes is believed to contribute the matrix accumulation.

**Study Design:** Study tried to find out the renal tubular proteins that may detect earlier phase of renal complication in diabetes.

**Material and Methods:** 42 female patients and 27 male patients using insulin (18-20 units) age range 40-60 yrs with longstanding diabetes, were included in the study. The patients were taken from the medical ward/ out door department of Sir Ganga Ram Hospital between Feb 2006 and Feb 2007. The electrophoretic patterns of 24 hr urinary proteins in diabetic patients were examined on 10 % SDS gradient polyacrylamide gel electrophoresis. 24 hr urinary protein of patients was estimated.

**Results:** Electrophoretic profile showed a condition of proteinuria (both high molecular weight and low molecular weight). It is observed that proteins with molecular weight ranges 120-28 Kda was appeared in urine samples of patients. While in normal subjects the range of the urinary protein is from 37-72 Kda. Among these the common proteins observed in patients and control urine sample was of protein of molecular weight 27 (alpha 1 microglobulin) and 42 Kda (acid glycoprotein). A significantly increased raw volume (concentration) and density of these 2 proteins was observed in urine sample of patients.

**Conclusion:** The technique of electrophoresis may be able to exclude or detect tubulo-interstitial nephropathies in earlier phases of renal complications, such as in diabetes mellitus.

**Key Words:** Diabetes Type 11, Alpha-1-acid glycoprotein, SDS electrophoresis

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### INTRODUCTION

Diabetic nephropathy is characterized by accumulation of mesangial matrix. It is observed that glucose-induced inhibition of matrix-degrading enzymes such as collagenases is believed to contribute to matrix accumulation. Furthermore, glucose-induced inhibition of MMP-2 (matrix metalloprotease) activity was completely blocked by neutralization of transforming growth factor (TGF)-beta<sup>1,2</sup>.

Detection of renal tubular proteins and enzymes in the urine demonstrated a tubular involvement that lead to renal complications of diabetes<sup>3</sup>. Serum and urinary markers (both glomerular and tubular) of diabetic nephropathy, play an important role in the early detection of renal damage<sup>4</sup> (Hong and China 1998). The markers of glomerular and tubular include (1) glomerular---transferrin, fibronectin, immunoglobulin G, Tamm-Horsfall protein and other components of glomerular extracellular matrix<sup>4,5</sup>, and (2) tubular--low molecular weight proteins (beta 2 microglobulin, retinol binding protein, alpha 1

microglobulin & alpha-1-acid glycoprotein). It is found that albumin served as "glomerular" and alpha 1-microglobulin as "tubular" markers measured by turbidimetry<sup>4,6,3</sup>.

Pathological changes in the urine sodium dodecyl sulphate gel electrophoresis (SDS PAGE) patterns often precede the occurrence of any sign of renal involvement in diabetes<sup>7</sup>. The typical urinary protein spectrum contained immunoglobulin G, Tamm-Horsfall protein, transferrin, albumin, beta 2-microglobulin (beta 2m), immunoglobulin light chains, retinol-binding protein, and alpha 1-microglobulin (alpha 1m). Low-molecular-weight proteinuria demonstrate the characteristic for the early stages of Balkan end nephropathy in diabetic patients. The experimental study showed that proteinuria in newborn, young and adult rats<sup>5</sup> is predominantly tubular, consisting of low molecular-weight species. Conversely, late adults and old rats had a mixed glomerular pattern, with a steadily increasing excretion of albumin, IgG and transferrin, as was the case of other high molecular-weight proteins<sup>8</sup>.

## PURPOSE OF STUDY

Present study tried to evaluate the urinary proteins as a marker of early renal tubular damage in diabetes mellitus using the technique of gel electrophoresis.

## MATERIAL AND METHODS

42 female patients and 27 male patients taking insulin (18-20 units), age range 40-60 yrs with longstanding diabetes, were included in the study. The patients were taken from the medical ward of Sir Ganga Ram Hospital between Feb 2006 and Feb 2007. Ten male and 10 female subjects of same age with no history of diabetes were considered as normal controls. The electrophoretic patterns of 24 hr urinary proteins in diabetic patients were examined on 10 % SDS gradient polyacrylamide gel electrophoresis<sup>9</sup>. Densitometric measurements were used to characterize protein excretion patterns patients. 24 hr urinary protein of patients was estimated with standard kit method.

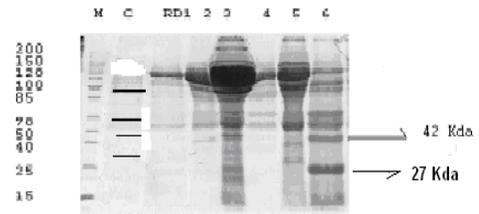


Fig: Photograph showing serum fractions of markers (M) range 200-15 Kda, serum fractions of proteins (C) and serum fractions of patients (2-6)

Table1: Mean age and 24 hr urinary protein of male and female patients with chronic diabetes. Values expressed as mean±SD.

Parameter	Male patients (29)	Female patients (42)	Normal Male (10)	Normal Female (10)
Age (yr)	49.06±11.83	52.89±9.04	44.90±9.10	50.28±10.8
Urinary Protein (gm/24 hr urine)	0.84±0.50**	0.81±0.65**	0.16±0.12	0.17±0.56
Blood sugar (mg/dl)	249.00±36.42**	259.00±40.42**	140.00±28.2	145.00±25.2

\*\*P>0.001= Highly significant difference

Table 2 : Average raw volume and density of patients and normal control subjects.

Parameters	Male Patients (29)	Female patients (42)	Normal male (10)	Normal Female (10)
Average raw vol of AGP	5020.42	5979.68	162.65	160.66
Average density of AGP	0.00809	0.00664	0.25466	0.2657
Average raw vol of AIM	6741.8	6078.10	69.57	65.88
Average density of AIM	0.00428	0.00525	0.99425	0.9247

## RESULTS

Mean age and 24 hr urinary protein and blood sugar of male and female patients with chronic diabetes were tabulated. It is observed that the mean age of male patient with chronic diabetes was 49.06 years while of female patients was 52.89 years. The levels of urinary protein in male and female patients were 0.84 and 0.81 gm/24 hour urine sample. Where as the level of urinary protein of normal male and female subjects were 0.16 and 0.17 gm/24 hour urine sample respectively. When the levels of urinary proteins of male and female patients were compared with normal male and female controls, it shows a highly significant difference (P<0.001).

The levels of blood sugar in male and female patients were 249 mg/dl and 259mg/dl respectively. Where as the level of blood sugar of normal male and

female subjects were 140.0 and 150.0 mg/dl respectively. When the levels of blood sugar of male and female patients were compared with normal male and female control subjects, it shows a highly significant difference (P<0.001).

Electrophoretic profile of patients of both sexes showed proteins with a molecular weight range from 26.00 KDa to 120 KDa with a density range of 0.004 to 0.274. Whereas in normal subject, the protein with a molecular weight range of 37.00 to 71.99 KDa with a density range of 0.109 to 0.585 (data not shown). The most common proteins in patients and normal subjects were of alpha 1 acid glycoproteins and alpha 1 microglobulin. It is observed that the raw volume (concentration) and density of alpha1-acid glycoprotein and of alpha 1 microglobulin was

increased in patients as compared to normal subjects.

## DISCUSSION

Urinary protein patterns were found to be useful to predict the high risk group for diabetic nephropathy in the preclinical stage. The protein pattern also discriminate nephropathic types of glomerular or tubular origin. These are also useful for clinicians to know the risk stage and prognosis for diabetic nephropathy<sup>10</sup>.

Level of 24 hr urinary protein was estimated in patients of both sexes with chronic diabetes. It was observed that the levels of urinary proteins of male and female patients were compared with normal male and female controls, it shows a highly significant difference ( $P < 0.001$ ). A study observed the additional proteins in urine samples of diabetes patients. Study instituted that these proteins can be used as markers for specific and accurate clinical analysis of Diabetic nephropathy<sup>11</sup>. It is reported that the pattern of proteinuria in group of diabetic patients may reflect hyperfiltration as well as tubular injury<sup>12</sup>.

Electrophoretic profile of patients of both sexes showed proteins with a molecular weight range from 26.00 KDa to 120 KDa. Whereas in normal subject, the protein with a molecular weight range of 37.00 to 71.99 KDa. The profile showed that in chronic diabetes there is a condition of proteinurea (both high molecular weight and low molecular weight), which may cause to excretes the protein which normally not appear in normal protein. The study is in accord with the study<sup>7,13</sup>. who used the technique of SDS-PAGE to study the pathological changes in the protein pattern of urine. Their results support the view that early stages of diabetic nephropathy may involve both glomerular and tubular dysfunction. However the exact clinical and prognostic significance of the information provided by SDS PAGE analysis remains to be elucidated. On the other, the findings<sup>5</sup> of a study demonstrate the low-molecular-weight proteinuria characteristic for the early stages of nephrology.

Raw volume (concentration) and density of alpha1-acid glycoprotein and of alpha 1 microglobulin in male, female and normal subjects was noted. It is observed that raw volume (concentration) and density of alpha1-acid glycoprotein and of alpha 1 microglobulin was greater in patients as compared to normal subjects. A group of workers<sup>14</sup> compared the level of AGP in healthy and normal subjects and they observed a high level of AGP in patients as compared to the normal subjects. Their study suggest that a high level of alpha-1-acid glycoprotein showed tissue damage. A group of workers<sup>15</sup>

observed markedly increased excretion of all low molecular weight protiens including alpha 1 microglobulin in group of diabetic patients. It is postulated that alpha-1-Microglobulin is useful in the detection of renal tubular damage in patients with outflow disease of the upper tract

It is therefore suggested that traditional urinalysis should be replaced by quantitative determination (SDS-PAGE) of urinary proteins . This recommended strategy is able to exclude or detect tubulo-interstitial nephropathies in earlier phases of renal complications, such as in diabetes mellitus.

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