ORIGINAL ARTICLE

ALPHA-1 MICROGLOBULIN: A MARKER FOR EARLY DETECTION OF TUBULAR DISORDERS IN DIABETIC NEPHROPATHY

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Background: Tubular damage as suggested by tubular proteinuria is a recognised feature of glomerulonephritis. The objectives of the study were to compare the level of α-microglobulin in normal and diabetic patients, and also to find out whether the level of α-1 microglobulin could become a laboratory marker for tubulo-interstitial damage in diabetic nephropathy. **Methods**: Twenty-nine registered Type II diabetic patients of either sex were studied. The patients' age ranged from 41–50 years who were admitted in the medical ward, and those who visited the outdoor department of Sir Ganga Ram Hospital, Lahore were included in the study. The duration of study was one year from June 2006 to June 2007. Ten normal subjects with no history of diabetes were taken as controls. Blood samples and 24 hour urine samples of patients of all groups were collected. The levels of urinary protein and blood sugar were estimated by auto analyser. Proteinuria positive urinary samples were analysed by SDS-PAGE electrophoresis. **Results**: The level of α-1 urinary protein was significantly increased in the group of diabetic patients as compared to the urinary protein level of normal subjects. Blood sugar level was also significantly increased in patients as compared to controls. Level of low molecular weight protein α-1 microglobulin showed an electrophoresis band of 28 Kda with an average volume of 6741.88 in the urine sample of patients. On the other hand, a very light, hardly recognisable band was observed in normal subjects. **Conclusion:** Urinary α-1 microglobulin provides a non-invasive and inexpensive diagnostic alternative for the diagnosis and monitoring of urinary tract disorders, i.e., early detection of tubular disorders of diabetic nephropathy. We propose that SDS-PAGE electrophoresis is a comparatively inexpensive diagnostic approach to detect this marker in the urine sample.

Keywords: Alpha-1 microglobulin, diabetic nephropathy, marker, urinary

INTRODUCTION

Urinary microproteins are becoming increasingly important in clinical diagnostics. They can contribute in the non-invasive early detection of renal abnormalities and the differentiation of various nephrological and urological pathologies.[1] A permanent and quantitatively abnormal proteinuria [>150 mg/24 hour] found in the laboratory should be followed by an identification and quantification of all proteins present. A biomarker is defined as a biologic characteristic that is measured and evaluated objectively as an indicator of normal biologic processes, pathogenic processes or pharmacologic response to therapeutic intervention.[2] Global electrophoretic techniques are the reference methods for the study of urinary proteins.[3]

Nephropathy of non-insulin dependant diabetes mellitus (NIDDM) is the most common cause of end stage renal failure (ESRF) in both developed and developing countries.[4] In most cases the long term glomerulonephritis course led to chronic renal failure. The histological abnormalities seen in patients with progressive renal failure consists of glomerulosclerosis and tubulo-interstitial nephritis. The changes in tubulointerstitial segment may lead to glomerular and vascular injury. Measurement of urinary excretion of low molecular weight proteins including alpha-1 microglobulin (A1M) and retinol-binding protein (RBP) was a valuable supplement in estimation of tubulo-interstetial system malfunction. These proteins are readily filtered by normal glomeruli and virtually completely reabsorbed by normal proximal tubules.[5]

Alpha-1 microglobulin has a yellow brown colour. It is heterogenous in size and charge. This is caused by an array of small chromophore prosthetic groups, attached to amino acid residues at the entrance of the lipocalin pocket. Agene in the lipocalin cluster encodes α-1 microglobulin together with a Kuntz-type proteinase inhibitor, bikunin.The gene is translated into the alpha-1 microglobulin-bikunin precursor, which is subsequently cleaved and the two proteins secreted to the blood separately.[6]

A1M is a 27-kDa glycoprotein also called protein HC, present in various body fluids. It is also found in blood and in connective tissue in most organs. It is most abundant at inter faces between the cells of the body and the environment, such as in lungs, intestine, kidneys and placenta, possessing immunosuppressive properties. As an immuno-modulatory protein, A1M seems a promising marker for evaluation of tubular function.[7]

The study was carried out in order to compare the level of α-microglobulin in normal and diabetic patients, and also to find out whether the level of α-1

microglobulin could become a laboratory marker for tubulo-interstitial damage in diabetic nephropathy.

MATERIAL AND METHODS

Twenty-nine registered Type II diabetic patients of either sex with no other renal pathology were included in the study. The patients' age ranged from 41–50 years, who were admitted in the medical ward or visited the outpatients department of Sir Ganga Ram hospital, Lahore. Duration of study was a period of one year (from June 2006 to June 2007). Normal subjects were 10 with no history of diabetes or any renal disease**.**

Blood samples and 24 hour urine samples of patients of all groups were collected. Levels of urinary protein and blood glucose were estimated by auto analyser. Proteinuria positive urinary samples were analysed by SDS-PAGE electrophoresis.

RESULTS

Comparison of biochemical and other parameters in different age groups of diabetic patients with normal subjects was tabulated. It was observed that the mean age of patients was 49 years while those of the controls were 45 years. The level of urinary protein was significantly increased (p>0.001) in the patient group as compared with the urinary protein level of normal subjects. Blood sugar level was also significantly increased (p>0.001) in patients as compared to normal subjects. The level of low molecular weight protein α-1 microglobulin showed an electrophoretic band of 28 Kda with an average volume of 6741.88 in the urine sample of patients. On the other hand a very light, hardly recognisable band was observed in normal subjects. (Table-1, Figure-1)

Table-1: Comparison of biochemical and other parameters in different age group of diabetics with normal subjects (Mean±SD)

Parameter	Patients (n=29)	Normal (n=10)
Age (Yr)	49.06±11.83	44.90±9.10
Urinary protein (gm/24 hr urine)	0.84±0.50	0.16±0.12
Blood sugar (mg/dl)	249.00±36.42	1140.00±28.2
Alpha-1 mocroglobulin (raw volume)	6741.88	150.90

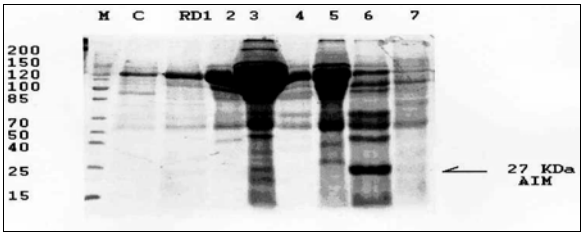

Figure-1: Urine protein profile of subject (from left to right) as separated on 12% resolving gel. Molecular markers are on extreme left from 200–15 Kda

C=control. RD 1–7=renal dysfunction of diabetics, M=molecular weight

DISCUSSION

The present study compared the biochemical and other parameters in diabetics and normal subjects. The level of urinary protein was significantly increased in the group of diabetic patients as compared with the urinary protein level of normal subjects. According to a study, diabetic patients with a degree proteinuria were the strongest determinants of faster glomerular filtration rate decline.[8] Study by another group stated that diabetic nephropathy was a frequent cause of end stage renal failure.[9] Our study is in accordance with other studies.[10] Data obtained from Proteomic analysis of the whole kidney do not provide any information regarding localisation. Therefore proteomic analyses for individual intrarenal proteomes are needed to better understand renal pathophysiology.[11] A group of workers observed a markedly increased excretion of all low molecular weight proteins including A1M in a group of diabetic patients.[7] It is postulated that A1M is useful in the detection of renal tubular damage in patients with outflow disease of the upper tract. The results of another group stressed the importance of tubular parameters such as A1M during early diabetes mellitus type 1 since they may serve as early markers of renal dysfunction and may precede albumin excretion.[12] Albuminuria measures glomerular dysfunction, where as A1M identified in diabetic patients can be used as markers for specific and accurate clinical analysis of tubulonephropathy.[13] Urine proteome analysis is a difficult task. Urine has low protein concentration, high levels of salts or other interfering compounds and high degree of variations.[14] There are several limitations of the use of microalbuminuria as an index of renal function. It is therefore desirable to identify additional protein markers that would augment prediction of diabetic nephropathy.

A1M is stable at low urinary *p*H. The high sensitivity of increased urinary excretion of this protein makes it an ideal instrument for demonstration of proximal tubular disorders.[9] The presence of A1M in urine is indicative solely of reduced reabsorptive capacity of proximal tubule because it is freely filtered through the glomeruli. Hence A1M is a better marker of proximal tubular dysfunction.

The present study observed a significant increase in the blood sugar level of diabetic patients which indicates their poor glycaemic control. Our study correlates with a study of workers who observed poor glycaemic control amongst diabetic patients. Weber MH reported that due to poor glycaemic control neuropathy, retinopathy and microalbuminuria are common.[5] Another study reported that increased urinary excretion of A1M and retinol binding protein reflected proximal tubular dysfunction in diabetic patients. However, only A1M correlated with the glycaemic control. It remains

to be determined whether this protein could serve as an additional early marker of diabetic nephropathy.[8]

CONCLUSION

Urinary A1M provides a non invasive, inexpensive diagnostic alternative for the diagnosis and monitoring of urinary tract disorders, i.e., early detection of tubular disorders of diabetic nephropathy. We propose that SDS-PAGE electrophoresis is a cost effective diagnostic approach to detect this marker in the urine sample

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