URINARY ENZYMES IN NEPHROTIC SYNDROME

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ABSTRACT

Tubular damage is a complication associated with nephrotic syndrome and increased levels of urinary enzymes are of significant value in detection of the same. The aim of our study was to evaluate the use of urinary lysozyme and trehalase as markers of tubular dysfunction in nephrotic syndrome. This study assessed 35 nephrotic syndrome patients and 30 healthy controls matched for age and sex. Urine samples were examined at pretreatment and post treatment (8 weeks) stages for proteinuria, lysozyme and trehalase. At pretreatment stage there was significant increase in urinary lysozyme and trehalase as compared to controls ($p < 0.001$). A good correlation was observed between degree of proteinuria and urinary lysozyme ($p < 0.001$; $r = 0.80$) and trehalase ($p < 0.001$; $r = 0.74$). At the end of 8 weeks of treatment, the patients showed significant decrease in their urinary lysozyme and trehalase activity ($p < 0.001$) but no correlation with degree of proteinuria was observed. Our results indicate that enzymes like lysozyme and trehalase can be used as markers of tubular dysfunction.

KEY WORDS

Lysozyme, Nephrotic syndrome, Trehalase.

INTRODUCTION

In Nephrotic syndrome, the presence of tubular abnormalities is considered as the most important determinant in progression of renal diseases. Estimation of urinary enzymes has been proposed to be of significant value in detection of tubular dysfunction. Urinary lysozyme and trehalase are amongst the enzymes studied.

Lysozyme is a basic protein of low molecular weight (14,800 Daltons) present in high concentration in kidney than any other mammalian tissue. As a low molecular weight protein lysozyme is almost completely reabsorbed by proximal tubules but little, if any, lysozyme activity is found in the urine per se (1). Lysozymuria has been reported in some children with nephrotic syndrome (2). The appearance of lysozyme in the urine in different syndromes of renal tubular dysfunction such as cadmium poisoning (3) and fanconi syndrome (4) has been suggested to be

because of failure of proximal tubular reabsorption of filtered proteins due to renal tubular damage. It has also been suggested that lysozymuria reflects pathological permeability of the lysosomes of the renal tubular cells.

Trehalase is an enzyme that hydrolyses trehalose (α -D-glucopyranosyl- α -D-glucopyranoside) to two glucose molecules and has been demonstrated to be localized only in the brush border of the intestine and renal proximal tubules (5). Although trehalase is believed to play an important role in sugar transport (6), the physiological role of the enzyme in the kidney is still unclear. Urinary trehalase has been reported to be increased in mercuric chloride-induced nephrotic rabbits (7), patients with the itai- itai disease and inhabitants of a cadmium polluted area (8, 9).

In cadmium poisoned patients urinary trehalase activity is found to be significantly low at the later stage of the disease due to reduced tubular cell mass. Study of urinary trehalase has been done as an indicator of tubular damage in newborn infants and infants treated with drug which causes tubular damage (10)

Little information is available on the value of these tests for detecting involvement of tubular dysfunction in nephrotic syndrome patients.

In this study our aim was to evaluate the use of urinary lysozyme and trehalase in detection of tubular damage in nephrotic syndrome.

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MATERIALS AND METHODS

Study participants were patients from nephrology OPD in the age range from 20-56 years, 20 M/15 F. Nephrotic syndrome was defined by proteinuria of greater than 3.5 g/day or urine protein/creatinine ratio above 3.5. Total of 35 nephrotic syndrome patients were included in the study and 30 healthy individuals of similar age and sex were studied as control.

All nephrotic syndrome patients were on steroid treatment for 8 weeks. Random urine samples were collected from patients before and after 8 weeks of treatment. Urine samples were used for determination of protein, creatinine, lysozyme and trehalase. Proteinuria was determined by turbidimetric test, urinary creatinine was determined by Jaffe's method. Protein excretion was expressed as ratio to urinary creatinine. Urinary lysozyme was determined by the spectrophotometric method of J.F. Harrison (4). The assay is based on the lysis of *Micrococcus lysodeikticus* cells by lysozyme. The values were expressed as µg/ml. Trehalase activity was assayed by the method of Sasai-Takedatsu with modification (10). The assay was based on the hydrolysis of trehalose by enzyme trehalase into two glucose molecules. The usual procedure of passing urine through Sephadex G 25 M (PD-10) column to eliminate background levels of glucose was avoided by taking control tubes. A set of tubes for unknown and control were taken; in both the tubes 0.9 ml of urine was added. To unknown tube 0.1 ml of 0.25 M trehalose (in phosphate buffer, pH 6.2) was added whereas, in control tube 0.1 ml of distilled water was added. Mixtures in both the tubes were incubated at 120 minutes at 37°C.

The glucose thus formed was determined by glucose determination kit (GOD- POD method). The observed increase in the concentration of glucose in the unknown tubes as compared to control tubes gave the activity of trehalase present in the urine sample. Trehalase activity was expressed as micromoles of glucose liberated per hour per liter of urine (µmol/h/l).

Statistical analysis

Mann-Whitney U test, *t* test and linear correlation were used for statistical analysis. Data are reported as mean±SD; *p* values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Elevations of urinary enzyme activity have been reported in a variety disease states in which proteinuria is a frequent if not constant manifestation. It has been shown that the degree of proteinuria correlates with proximal tubular cell injury and that reabsorbed proteins and congestion of proximal

tubular cells precede interstitial changes (11). It is of great interest to investigate whether enzymes like trehalase and lysozyme provide information about the severity of tubular damage in nephrotic syndrome. Table no 1 shows urinary P/Cr ratio, urinary lysozyme and trehalase activity of patients with nephrotic syndrome and controls. The results are expressed at pretreatment and at posttreatment. In our study, nephrotic syndrome patients showed heavy proteinuria, which significantly decreases after 8 weeks of treatment.

Lysozymuria

Lysozyme is easily filtered by glomerulus and almost completely reabsorbed by tubular cells. It is usually absent or present in only trace amounts in urine of human subjects, but is excreted in some patients with renal diseases (12,13) In the present study, lysozymuria was found to be significantly increased in nephrotic syndrome patients as compared to controls (*p* < 0.001). The increased activity was seen in 68.5 % of patients. The lysozyme found in the urine of nephrotic syndrome patients may originate from tubular cells that are damaged during the destruction of renal tissue. Peritubular macrophages are also a possible source of urinary lysozyme since macrophages have been shown to contain large amounts of the enzyme (13). As can be seen from mean values there was a significant decrease in post treatment level of urinary lysozyme in most of the nephrotic syndrome patients (*p* < 0.001).

The present study suggests that the detection of lysozymuria may be of significant value in the evaluation of tubular dysfunction as number of patients were noted to have severe to moderate lysozymuria.

The presence of lysozyme in the urine of patients with renal diseases has previously been reported but the relation of lysozymuria to renal damage has not been studied. Studies by Piel et al showed little increase in lysozyme excretion when the nephrotic syndrome was produced with an antibody preparation that resulted in glomerular damage (14). By contrast, marked lysozymuria was observed in experimental animals after the administration of mercuric chloride, which produced tubular lesions without significant glomerular damage (15).

The observations made in these studies are all consistent with the conclusion that lysozymuria is the result of renal tubular damage.

Urinary Trehalase

Trehalase is demonstrated to be located only in the brush border membrane of renal proximal tubules and the small intestine (5). In present study as seen from Table 1

Table 1. Urinary lysozyme and trehalase in nephrotic syndrome and healthy individuals

	Controls (N = 30)	Nephrotic syndrome (N = 35)	
		Pretreatment	Post treatment
Urine protein/ creatinine Ratio	-	7.55 ±3.89*	1.55±1.33*
Urinary lysozyme (µg/ml)	0.98 ±0.31	5.53±5.15*	1.34±0.87*
Urinary trehalase (µmol/h/l)	23.6 ±10.7	91±56.4*	34.5±18.9*
Values are expressed as mean±SD; * $p < 0.001$			
Statistical comparison was done between controls and nephrotic syndrome patients, Pre and Posttreatment levels of nephrotic syndrome patients.			

urinary trehalase activity was found to be significantly elevated as compared to controls ($p < 0.001$) and was noted in 62.8 % of patients. Studies by Niwa *et al* showed significant increase in urinary trehalase activity in patients with Nephrotic syndrome, chronic glomerulonephritis and chronic renal failure (16). Among these, patients with nephrotic syndrome were shown to exhibit high trehalase activity followed by those with chronic glomerulonephritis. Niwa *et al.* (16) proposed a mechanism for enhanced urinary trehalase activity in nephrotic syndrome as follows. A large amount of proteins that have passed through the glomerular basement membrane might lead to damage of renal proximal tubular cells and then trehalase might escape from the brush border of renal proximal tubules into urine. Since trehalase is located only in the brush border membrane of proximal tubules, it has been suggested to be more specific for renal brush border damage (16).

In our study, significant decrease in trehalase activity was demonstrated in all nephrotic syndrome patients at the end of 8 weeks of treatment, showing that trehalase activity is integral part of tubular cells.

Correlation study

It has been established that degree of proteinuria correlates well with tubulo- interstitial damage and progression to chronic renal failure (17,18). It is of interest to see whether this proteinuria also correlates with enzymuria specifically lysozyme and trehalase so that renal tubular cell damage can be identified.

In the present study, a strong correlation between degree of proteinuria and lysozymuria was seen only at the basal level (pretreatment) ($p < 0.001$; $r = 0.80$) as shown in Fig. 1 whereas at posttreatment no correlation was seen.

This indicates that lysozymuria could be a good indicator of the disease process in acute condition and may be a possible marker of functional outcome of

Fig. 1 Relationship between urinary lysozyme and urinary protein/ creatinine ratio in nephrotic syndrome patients at pretreatment level

renal tubular cells. The lack of correlation at posttreatment stage indicates the improvement in tubular function.

As regards urinary trehalase, good correlation was seen between degree of proteinuria and trehalase at pretreatment level ($p < 0.001$; $r = 0.74$) as can be seen from Fig. 2 while no correlation was seen at post treatment level.

Caliskan *et al.* (19) in their study about urinary N-acetyl- β -D-glucosaminidase and β_2 microglobulin in nephrotic syndrome observed elevation in the excretion of both of these during the relapse phase and decreasing to the normal range in remission. They concluded that this might be related to the transient proximal tubular damage and dysfunction in nephrotic syndrome. Our results are consistent with studies of Caliskan *et al.* (19) indicating transient tubular damage and dysfunction.

Fig. 2 Relationship between urinary trehalase and urinary protein/ creatinine ratio in nephrotic syndrome patients at pretreatment level

If both urinary trehalase and urinary lysozyme are taken as the indicators of tubular damage, one could differentiate between morphological damage and dysfunction of reabsorption of tubular cells. Urinary trehalase and lysozyme show different aspects of tubular damage: the former shows a direct morphological damage at tubular brush borders, and the latter shows a dysfunction of reabsorption at tubular sites (20). This indicates that urinary trehalase more closely reflects structural abnormalities of tubules, whereas urinary lysozyme reveals functional damage caused to the tubules.

In our study at pretreatment, since the severity of tubular damage is quite high, there is correlation between proteinuria and enzymuria. After treatment, although there is decrease in degree of proteinuria, it is still seen to persist to some extent while not correlating with enzymuria.

Since transient tubular damage is a common phenomenon in nephrotic syndrome, additional tests like estimation of urinary enzymes such as trehalase and lysozyme are suggested, which could highlight the extent of tubular damage involvement in nephrotic syndrome.

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