Urinary lysozyme levels in primary nephrotic syndrome and amyloidosis

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Urinary lysozyme activity mainly reflects tubular injury. Urinary and serum lysozyme levels along with other routine measures were assessed in normal controls, eight idiopathic nephrotic syndrome and 13 renal amyloidosis patients. Using urinary lysozyme (Lzm), serum Lzm and urine Lzm/urine creatinine values as independent variables a Y value was defined to discriminate between amyloidosis, idiopathic nephrotic syndrome and normals. When four additional variables were included (serum protein, albumin, urine protein/urine creatinin and urine Lzm/urine protein ratios) another Y value was defined to discriminate between amyloidosis and idiopathic nephrotic syndrome cases. Thus these measurements may be used in selected cases to identify idiopathic nephrotic syndrome patients before performing a renal biopsy and save the amyloidosis patients from the deleterious effects of corticosteroid treatment. [Turk J Med Res 1993;11(5): 237-239]

Key Words: Nephrotic syndrome, Amyloidosis, Lysosyme

The increased urinary excretion of N-acetyl-beta-glucosaminidase (NAG), alanine aminopeptidase (AAP), lysozyme (Lzm) and similar enzymes are valuable markers in reflecting renal damage and especially "injury" to the renal tubule (1-3). The urinary enzyme activity may originate from the renal parenchyma, urogenital tract or plasma. Under normal circumstances only a slight amount of the enzymes are filtered by the glomerulus and they are almost completely reabsorbed in the proximal renal tubule (3). Although increased excretion of these enzymes may be observed in glomerular permeability disturbances, even along with nephrotic syndrome, such increment is inferred to reflect tubular damage or interstitial damage (4-8). Since amyloidosis is known to result in tubulointerstitial accumulation of amyloid material lysozymuria may be expected to be more pronounced in renal amyloidosis patients when compared to some glomerular diseases.

In this study serum and urine lysozyme concentrations have been studied in idiopathic nephrotic syndrome and in amyloidosis patients in an attempt to analyze whether they can be used in the differential diagnosis of these two diseases.

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MATERIALS AND METHODS

This study has been conducted in three groups. Group I was consisted of 13 renal amyloidosis patients with an age range of 9 to 18. Group II was consisted of 8 idiopathic nephrotic syndrome (NS) patients with an active disease and an age range of 4 to 17. Group Ⅲ was consisted of 10 age and sex-matched healty patients with normal urinalysis findings.

In the amyloidosis and idiopathic nephrotic syndrome patients with normal renal functions, serum creatinine, protein, albumin, Lzm levels, as well as urine protein, creatinine, Lzm levels were measured and urine protein/urine creatinine (Up/Ucr), urine Lzm/urine creatinine, urine Lzm/urine protein ratios were calculated for these patients. In the control group urine Lzm/urine creatinine ratio was calculated for each patient.

Serum and urine Lzm activities were assessed by turbidimetry as previously described (9). Enzyme standards (0.01-2.50 mg/l) and a Micrococcus lysodeikticus suspension (200 mg/l) were freshly prepared in Sorensen's phosphate buffer. Assay mixtures containing 2.5 ml M.lysodeikticus suspension and 250 pi aliquots of buffer blank, standards or samples all induplicate, were incubated at 37° C for 5 min and absorbances were measured at 570 nm. Serum lysozyme values were expressed as mg/l. Urinary lysozyme values were reported both in absolute concentration (mg/l) and in relation to creatinine content

(ug/mg creatinine). Creatinine concentrations were determined as previously described.

The variables of the collected data have been analyzed for the three groups by non-parametric tests. The analysis was an application of the linear discriminant function. For the first two groups arithmetic mean, standard deviation and correlations were evaluated for seven selected variables. Pair-wise comparisons were made among the three groups in terms of the selected variables, taking account of their intercorrelations. The analysis as a multiple regression procedure, and an application of the linear discriminant function (10). An equation was derived for discriminating the patient groups.

RESULTS

The mean serum and urine Lzm values in the three groups are displayed in Table 1. The difference between the serum Lzm levels in these three groups were not statistically significant ($p>0.05$).

The urine Lzm/urine creatinine ratios in the amyloidosis, idiopathic nephrotic syndrome and control groups were 0.93±0.46, 1.27+0.47 and 0.09+0.02, respectively. The differences between the control group and both of the patient groups were statistically significant. Urine Lzm/urine protein ratios in the amyloidosis and idiopathic nephrotic syndrome groups were 0.12+0.04 and 0.10+0.03, respectively and the difference between these two groups was not statistically significant.

In neither of the groups there was no correlation between urine Lzm and protein levels.

Urine Lzm, urine Lzm/urine creatinine and serum Lzm values were selected as independent values X1, X2 and X3, respectively in an attemt to differentiate between the three groups of patients. Y values were identified by use of discriminant function:

Y 1 - 0.673596 X1 + 0.66057 X2 + 0.331533 X3

Y 2 - -0.188492 X1 - 0.280157 X2 + 0.941255 X3

When the means for each group were selected as the X variables, the points of discrimination for Y-Y1 + Y2 were 4.9985 and 5.8945. Thus it was observed that:

Y<4.9985.....case: normal

5.8945>Y>4.9985.....case: idiopathic NS

5.8945<Y.....case: amyloidosis

Selecting seven independent X variables listed in Table 2 we have identified another Y value (Table 2). By the use of this equation it was concluded that: (Y<2.87 indicated amyloidosis, and Y>2.87 indicated idiopathic NS.

Table 1. Serum and urine lysozyme activities

Patient Group	Serum Lysozyme activity	Urine Lysozyme activity
Amylodosis	4,25±0,51 mg/lt	0,57+0,25 mg/lt
Idopathic NS	3,86±0,45 mg/lt	0,70±0,36 mg/lt
Healthy control	3,30+029 mg/lt	0,06±0,09 mg/lt

Table 2. Independent variables

X1 Serum Protein
X2 Serum albumin
X3 Urine Protein/Urine Creatinine
X4 Urine Lysozyme/Urine Protein
X5 Urine Lysozyme/Urine Creatinine
X6 Urine Lysozyme
X7 Serum Lysozyme

Y——0,0438 X1 - 0,0814 X2 +- 0,2067 X3 - 0,5709 X4 - 0,1929 -0,5197 X6-05617X7

DISCUSSION

Lysozyme, a protein of low molecular weight, is readily filtered by the glomerulus and is reabsorbed in the proximal renal tubule where it is enzymatically degraded (12). The serum Lzm concentration mainly depends on glomerular filtration rate not on tubular function and consequently the concentration tends to rise parallel to advancing azotemia. The high serum Lzm levels observed in renal failure is due to inadequate excretion by the kidney along with insufficient catabolism within the tubular cells (4,7).

In our patient group the serum Lzm levels were normal. Since they all had normal renal function this was an anticipated result.

In cases of organic or functional tubular impairment lysozymuria is observed without a rise in serum Lzm due to diminished reabsorbtive capacity. Lysozymuria has been documentated in glomerulopathies with tubulointerstitial changes as well.

If renal injury is confined to the glomeruli, lysozymuria is absent. Prockop and Davidson (13) have shown that lysozymuria was negligible in glomerular injury induced by immonocomplex nephritis in animal studies, but when tubular injury was induced by HgCI injection lysozymuria has significantly increased. Marked lysozymuria has also been demontrated in proximal tubular disorders such as Lowe syndrome and cystinosis (6,14).

There have been reports correlating lysozymuria and histopathological findings in tubulointerstitial nephritis, in acute glomerulonephritis, focal nephritis, nephrotic syndrome and chronic glomerulonephritis (15). In yet another study it was suggested that, reversible lysozymuria in nephrotic patients may be related

either to renal edema or to a possible intracellular electrolyte imbalance (7).

In this study the urine Lzm levels and urine Lzm/urine creatinine ratios in amyloidosis and idiopathic NS patients were significantly raised when compared to the control group. On the other hand the difference between the amyloidosis and primary NS group was not statistically significant. The amyloid material is known to also accumulate within the medullary portion of the kidney. Consequently it causes various tubular disorders as well. Five of our idiopathic NS patients were diagnosed as focal sclerosing glomerulonephritis which may also cause tubular dysfunction.

Lysozymuria is defined as Lzm excretion exceeding 2 mg/lt (4). Three of our 13 amyloidosis cases had levels exceeding 2 mg/lt and these may be explained by the presence of tubular injury. In two of our idiopathic NS patients high urine Lzm levels may be explained by the presence of the focal sclerosing process in one and prerenal azotemia with peritubular edema in the other. Although the mean urine Lzm levels were increased both in the amyloidosis and the idiopathic NS groups, they were not above 2 mg/lt in the rest of the patients. Serum Lzm and urine Lzm/urine creatinine levels were not significantly different in the amyloidosis and idiopathic NS groups when they were analyzed separately. When these two variables along with urine Lzm levels were used, multivariate analysis permitted very good discrimination between normals, idiopathic NS and amyloidosis patients. The equation obtained from multivariate analysis of four additional variables yields accurate predictions of the underlying glomerular histopatholgy. If the Y value thus obtained is <2.87 this indicates amyloidosis and >2.87 defines idiopathic NS.

Thus this analysis has defined Y values of high confidence in differentiation of the two disease entities. This quantitative figure will enable the clinician to make a clear judgement. This will save certain amyloidosis patients from the deleterious effects of corticosteroids and may serve as a preliminary test before performing a biopsy.

Primer nefrolik sendrom ve amiloidosis de idrar lizozim düzeyleri

İdrar lizozim aktivitesinin tübüler hasarı gösterdiği kabul edilmektedir. Bu çalışmada yaş ve cins dağılımı benzer normal çocuklarda, sekiz idiopatik nefrotik sendrom (NS) ve 13 renal amiloidoz hastasında diğer rutin tetkiklerin yanısıra idrar lizozim (Lzm) ve serum Lzm düzeyleri ölçülmüştür, idrar Lzm, serum Lzm ve idrar Lzm/kreatinin değerleri kullanılarak normal çocuklar, idiopatik NS ve amiloidoz hastaları arasında ayrım sağlayacak bir Y değeri tanımlanmıştır. Bu üç parametreye ek olarak serum protein, albumin, idrar proteirVkreatinin ve idrar Lzm/protein oranları kullanılarak yine amiloidoz ve idiopatik NS vakaları arasında ayrım sağlayacak bir formül de tanımlanmıştır. Bu nedenle bu parametreler kullanılarak hesaplanacak değerler ile biyopsi öncesi idiopatik NS ve amiloidoz arasında güvenilir ayırıcı tanı yapılabilir.

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REFERENCES

1. Kunin CM, Chesney RW, Craig WA, et al. Enzymuria as a marker of renal injury and disease. Pediatrics 1978; 62:751-60.
2. Dance N, Price RC. The excretion of N-Acetly-B qlucosaminidase and beta qaloctos dase by patients with renal disease. Clin Chim Acta 1970; 27:87-92.
3. Al-Bander HA, Mock DM, Etheredqe SB, et al. Coordinately increased lysozymuria and lysosomal enzymuria induced by maleic acid. Kidney Int 1986; 30:804-12.
4. Hayslett IP, Perillie PE, Finch SC. Urinary muramidase and renal disease. New Enql J Med 1968; 279:506-17.
5. Zaqer RA. Urinary protein markers of tubulointerstitial nephritis. Invest Urol 1980; 18:197-202.
6. Barrat TM, Crowford R. Lysozyme excretion as a measure of renal tubular dysfunction in children. Clin Sci 1970; 39:457-65.
7. Harrison JF, Parker RW, De Silva KL. Lysozymuria and acute disorders of renal function. J Clin Path. 1973; 26:278-84.
8. Shima K, Hiroto M, Fukuda M, et al. Determination of urinary lysozyme for potential detection of tubular dysfunction in diabetic nephropathy. Clin Chem 1986; 32:1818-22.
9. Houser MT. Improved turbidimetric assay for lysozyme in urine. Clin Chem 1983; 29:1488-1493.
10. Morrison DF. Multivariate statistical methods (2nd ed). New York: Mc Graw-Hill Book Co. Willey 1962.
11. Conover WJ. Practical nonparametric statistic. New York: John Wiley and Sons Inc. 1980.
12. Harrison JF and Barnes AD. The Urinary excretion of lysozyme in doqs. Clin Sci 1970; 38:535-47.
13. Prockop DJ, Davidson WD. Study of urinary and serum lysozyme in patients with renal disease. New Enql J Med 1964; 270:269-74.
14. Fujita T, Itakura M. Renal handling of lysozyme in experimental fanconi syndrome. J Lab Clin Med 1978; 92:135-47.
15. Hultberq B, Raunskov U. The excretion of N-aecetyl-beta qlucosaminidase in qlomerulonephritis. Clin Nephrol 1981; 15:33-38.