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ENZYMURIA PATTERN IN EARLY POST RENAL TRANSPLANT PERIOD: DIAGNOSTIC USEFULNESS IN GRAFT DYSFUNCTION

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ABSTRACT

Serum creatinine does not distinguish between various causes of graft dysfunction. Serial assay of proximal tubular enzymes N-Acetyl-D-glucosaminidase (NAG), Alanine aminopeptidase (AAP) and Gamma glutamyl transferase (GGT) in urine was done to assess their usefulness in distinguishing various causes of graft dysfunction. Daily serum creatinine and enzymuria were measured in 32 consecutive renal allograft recipients for first 15 postoperative days. Graft dysfunction was defined as >20% increase in serum creatinine and >100% increase in enzymuria over the baseline. The diagnosis of graft dysfunction was based upon clinical criteria, ultrasonography, cyclosporin trough level, allograft biopsy, response to anti-rejection therapy and alteration of cyclosporin dosage. Fifteen episodes of graft dysfunction were identified in 15 patients. The sensitivity and specificity of the enzymes (NAG, AAP and GGT) for predicting graft dysfunction were 87.5%,86.9%,88.5% and 98.2%,98.2%,97.9% respectively. There was a significant increase in enzymuria during acute tubular necrosis (ATN) and acute rejection episode compared to cyclosporin nephrotoxicity ($p < 0.01$). Enzymuria assay provides a simple, reliable and noninvasive method to distinguish cyclosporin nephrotoxicity from acute tubular necrosis and acute rejection in renal allograft recipients.

KEY WORDS

Acute rejection, acute tubular necrosis, cyclosporin nephrotoxicity, renal transplantation, urinary enzymes.

INTRODUCTION

Serum creatinine, the commonly used marker of renal allograft dysfunction rises only after significant damage has occurred and does not allow distinction among various causes of graft dysfunction. N-acetyl glucosaminidase (NAG), Alanine aminopeptidase (AAP) and Gamma glutamyl transferase (GGT) are proximal tubular enzymes whose urinary levels have been reported to increase during episodes of renal damage (1-4). There is however a long standing debate regarding the relative time of increase of enzymuria (5-9)and serum creatinine and the capability of enzymuria to differentiate the various causes of graft dysfunctions in the post transplant period (9-11). Several authors have described enzymuria as an earlier marker of allograft dysfunction compared to creatinine (5-8) while others have failed to agree with that (9). However, a large clinical trial to verify the diagnostic specificity and sensitivity of enzymuria for earlier identification and differentiation of the cause of graft dysfunction episodes is still lacking.

The present prospective study was undertaken to ascertain the pattern of enzymuria in the immediate post transplant period, and its correlation with episodes of graft dysfunction in 32 consecutive living donor renal allograft recipients transplanted between October 1998 and May 1999.

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MATERIALS AND METHODS

Thirty-two consecutive living donor renal allograft recipients transplanted between October 1998 and May 1999 were included in this study.

All patients had serum creatinine and blood counts

monitored daily. Daily morning 10 ml urine samples were collected for estimation of enzymuria and stored at 4°C until analysis. Samples were analyzed daily from one day pre operatively to 15 days post operatively for GGT, NAG and AAP as well as urinary creatinine.

The levels of the urinary enzymes were determined by following the method previously reported by us (12). Ten ml of urine was centrifuged at 900 g for 10 minutes. One ml of the urine supernatant was loaded on Sephadex G25 column (Bead volume 5.6 ml, previously equilibrated with 0.15M NaCl solution). The enzymes were eluted out of the column with 2.5 ml of 0.15 M NaCl solution and stored at 0-4°C for analysis. Enzyme activities were measured in a semi automated spectrophotometer (BT 224, Biotechnica, Italy). GGT was analyzed by following the method of Jung *et al.* (13), whereas NAG and AAP were measured according to the method of Marhun, (14) and Jung and Schloz, (15) respectively. The serum & urinary creatinine was estimated by the Jaffe reaction on a fully automated clinical chemistry analyzer (Express plus, Ciba Corning). Enzyme activities were expressed as Units per gm of urinary creatinine.

Graft dysfunction was defined as 20% or more increase of serum creatinine over the baseline. Significant enzymuria was defined as greater than 100% increase over the baseline (1, 9, 11). The diagnosis of the cause of allograft dysfunction was based upon clinical criteria, ultrasonography, cyclosporin trough levels, allograft biopsy, response to anti-rejection therapy and alteration of cyclosporin dosage.

A concomitant increase in serum creatinine and enzymuria was defined as true positive. An increase in enzymuria not accompanied by an increase in serum creatinine was defined as a false positive. An increase in serum creatinine without a preceding, coinciding or subsequent increase in enzymuria was defined as a false negative result. No increase from the baseline in either serum creatinine or enzymuria was defined as a true negative result.

The sensitivity, specificity, positive predictive and negative predictive values of the enzymuria assay was determined by 2x2 cross-matrix analysis.

This study was approved by the hospital ethics committee.

RESULTS

Table 1 shows the mean pre-transplant urinary excretion levels of the enzymes GGT, NAG and AAP in the 32 patients with chronic renal failure during the present study. The levels were found to be higher by 2.8 fold for GGT, 7 fold for NAG and 3.7 fold for AAP as compared to the mean excretion levels of the corresponding enzymes in 76 healthy volunteers reported earlier (12).

Table 1. Urinary enzyme excretion levels in normal healthy volunteers and the pre transplant levels in the chronic renal failure patients

	Urinary enzyme excretion levels (U/gm of urinary creatinine)		
	GGT	**NAG**	**AAP**
Patients (n=32)	44.9 ± 35.7*	30.9 ± 30.0*	20.9 ± 11.8*
Healthy Volunteers (n=76)	16.17 ± 2.9	4.4 ± 1.1	5.6 ± 4.1

Data are expressed as the mean ± SD.

*P<0.001 for each enzyme of chronic renal failure patient group in comparison to the corresponding enzymes of normal healthy volunteers group

A total of 462 urine samples from 32 patients were analyzed. In 17 of the 32 patients enzymuria and serum creatinine reached a nadir and remained at the baseline levels until the patient was discharged from the hospital. The baseline of enzymuria was reached on average 3 days before that of the serum creatinine. Table 2 shows the daily mean excretion of the enzymes during the first 15 days of study after transplantation in 17 patients without any graft dysfunction episodes. In 15 patients, episodes of graft dysfunctions were noted. The causes of graft dysfunction were acute rejection 6, acute tubular necrosis 4 and cyclosporin toxicity 5. Ten biopsies were performed in 8 patients while in the rest of the patients the graft dysfunction patterns were validated by the clinical situation, cyclosporin levels and the response to a therapeutic intervention like altering the cyclosporin dose or treatment with anti-rejection therapy. In the 15 episodes of graft dysfunctions the increase in NAG preceded the increase in serum creatinine in all 15 episodes, whereas the increase in GGT and AAP preceded by 13 & 14 episodes. The increase in GGT & AAP coincided with the serum creatinine increase in 2 & 1 episodes respectively (Table 3).

Table 2. Post transplant enzymuria in renal allograft recipients without any dysfunction episodes

Post-transplant day	Urinary enzyme excretion levels (U/gm of urinary creatinine)		
	GGT	NAG	AAP
1	26.5±13.0	12.1±7.6	13.2±11.0
2	35.9±26.8	14.0±10.8	12.5±9.4
3	29.4±13.7	10.5±3.9	10.1±6.4
4	25.7±12.6	12.9±9.9	7.2±3.9
5	29.8±16.5	12.7±12.2	9.0±6.7
6	28.2±13.0	12.2±7.5	11.4±6.8
7	26.7±10.4	10.8±4.3	9.7±4.8
8	25.2±10.0	9.6±7.0	7.9±2.5
9	23.8±14.6	12.5±10.0	9.5±5.8
10	28.2±16.8	16.1±19.0	11.5±8.0
11	19.5±11.0	13.0±8.7	11.9±4.6
12	27.5±11.5	10.5±5.7	10.8±6.6
13	25.5±7.3	9.3±3.8	9.4±5.9
14	23.8±8.9	8.4±4.0	8.3±3.8
15	27.1±7.5	9.2±3.3	12.5±1.0

Data are expressed as the mean±SD of enzymuria levels of 17 patients in the 15 post surgical days

Table 4 shows that the sensitivity and specificity of GGT, NAG and AAP for predicting acute graft dysfunction was 88.5%, 87.5%, 86.9% and 97.9%, 98.2%, 98.2% respectively.

Fig. 1 to 3 shows the typical pattern of enzymuria in these renal allograft recipients who developed graft dysfunction in the post operative period.

Table 5 shows the degree of increase of the enzymes over the baseline during the different episodes of graft dysfunctions. There was 15.7 and 7.2 fold increase of NAG over the baseline in acute necrosis and rejection episodes. The GGT was increased by 7.6 and 5.3 folds. In cyclosporin toxicity episodes the degree of increase of GGT, NAG and AAP are only 2.1, 3.1 and 2.5 fold over the baseline levels which are significantly low ($p<0.01$) in comparison to the increase of the corresponding enzymes in both the acute rejection and acute tubular necrosis episodes. However, only the increase of NAG was found to be significantly ($p<0.05$) higher in the acute tubular necrosis

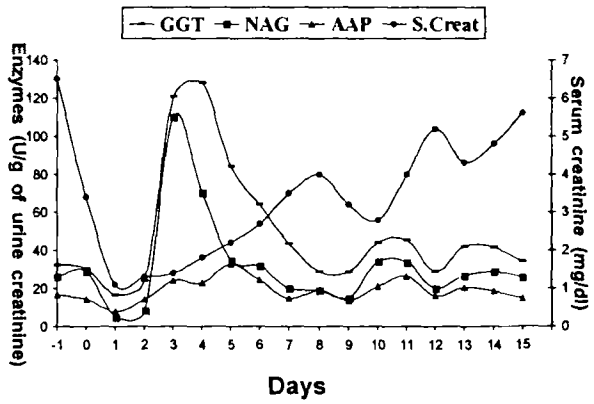

Fig. 1. Pattern of enzymuria in a 38-year female allograft recipient with acute tubular necrosis. The graft dysfunction was confirmed by graft biopsy (RBx) on post-operative day 5. Enzymes were reached their peaks before the serum creatinine was increased by 29% on day 4.

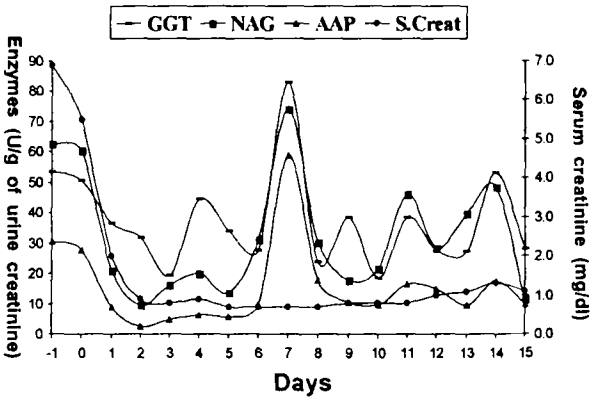

Fig. 2. The enzymuria pattern in a 43-year male recipient with graft biopsy (RBx) confirmed acute rejection episode. On day 12 the serum creatinine increased by more than 20% but the enzyme peaks were obtained clearly on day 7. A persisting daily fluctuation of enzymuria coinciding as well as following the increase of serum creatinine was noted in this episode of graft dysfunction.

episode as compared to the increase of the same in the acute rejection episode. Though the increase of GGT and AAP is higher in ATN compared to acute rejection, the increase is not statistically significant.

DISCUSSION

The mean of pre-transplant enzymuria from the 32 patients with chronic renal failure was significantly higher ($p<0.001$) as compared to 76 healthy volunteers. Our data corroborates with previous reports of increased urinary excretion of the brush

Table 3. Relative occurrence of rise of serum creatinine and enzymuria in the different episodes of graft dysfunction

Episodes of graft dysfunction	Number	Rise in enzymuria and serum creatinine					
		GGT		NAG		AAP	
		P*	C**	P	C	P	C
Acute rejection	6	4	2	6	0	5	1
Acute tubular necrosis	4	4	0	4	0	4	0
Cyclosporin toxicity	5	5	0	5	0	5	0
Total	15	13	2	15	0	14	1

*: Preceded occurrence
**: Coincided occurrence
$p<0.001$ for all the 3 enzymes over the baseline values in the case of both the preceded and coincided occurrence with respect to that of creatinine against all graft dysfunction episodes.

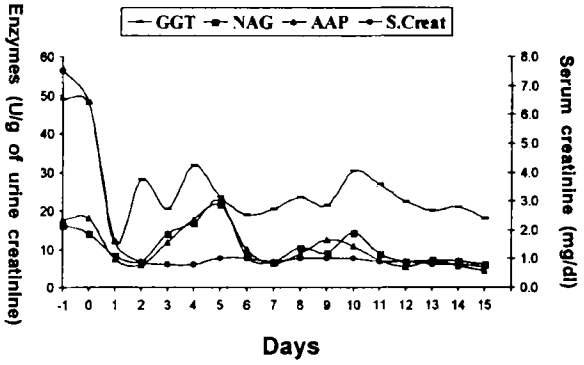

Fig. 3. Urinary enzyme pattern in connection with cyclosporin induced nephrotoxicity (High C_0 levels). The values are of a 18 year male renal allograft recipient included in the present study. A 25% increase of serum creatinine was noted on post-transplant day 5 following an enzymuria peak on day 3-4. The enzyme excretion levels here are characteristically low in comparison to the acute rejection and necrosis episodes.

border enzymes in patients with interstitial nephritis (16), Glomerulonephritis (17) and Diabetic nephropathy (18). Following living donor renal transplantation there is a rapid fall in enzymuria.

Conflicting reports are present regarding the relative occurrence of enzymuria during the episodes of

Table 4. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of enzymuria

	GGT	NAG	AAP
True positive (T+)	23[a]	21[a]	20[a]
False positive (D+)	9[b]	8[b]	8[b]
False negative (D-)	3[c]	3[c]	3[c]
True negative (T-)	427[d]	430[d]	431[d]
Sensitivity (%) : [a/(a+c)]x100	88.5	87.5	86.9
Specificity (%) : [b/(b+d)]x100	97.9	98.2	98.2
PPV (%) : [a/(a+b)]x100	71.8	72.4	71.4
NPV (%): [d/(c+d]x100	99.3	99.3	99.3

True positive (T+) : 100% increase in enzyme level and 20% increase in s.Cr.
False positive (D+) : 100% increase in enzyme level and no change in s.Cr.
False negative (D-) : No change in enzyme level and 20% increase in s.Cr.
True negative (T-) : No change in enzyme level and no change in s.Cr.

Table 5. Number of times of increase of the enzymes GGT, NAG and AAP over the corresponding baseline excretion levels in the different episodes of graft dysfunction as confirmed after renal biopsy

Episode of of graft dysfunction (Renal biopsy output)	No.	GGT	NAG	AAP
a. Acute tubular necrosis	4	7.6 ± 3.5	15.7 ± 6.4	3.9 ± 0.4
b. Acute rejection	6	5.3 ± 1.8	7.2 ± 4.0	3.8 ± 0.3
c. Cyclosporin toxicity	5	2.1 ± 0.3	3.1 ± 1.0	2.5 ± 0.5
$p<0.01$ for all the 3 enzymes in a Vs c $p<0.05$ only for NAG in a Vs b $p<0.01$ for all the 3 enzymes in b Vs c				

graft dysfunction in renal transplant recipients (9,10,11). Bornstein (9) reported enzymuria to increase concurrently or rather after the increase in serum creatinine in response to graft dysfunction, while some others reported it to precede an increase of serum creatinine (5-7). The most important findings of our investigation are that the increase in excretion of NAG preceded the serum creatinine in all 15 episodes of graft dysfunction, whereas the GGT and AAP preceded it in 13 and 14 episodes (Table 3).

We also found that the increase in enzymuria was several fold higher in patients with ATN and acute rejection compared cyclosporin toxicity. This probably is due to definite tubular cell damage in patients with ATN and acute rejection whereas in cyclosporin toxicity there is often drug induced functional ischemic renal dysfunction (10). The enzyme pattern during follow up shows a single peak followed by return to base line in ATN where as in acute rejection there is large peak followed by smoldering small rise and fall of enzymuria reflecting polyphasic injury (19).

Amongst the various enzymes studied NAG had the highest discriminatory power to distinguish between ATN, acute rejection and cyclosporin toxicity (Table 5). However, we feel that in clinical practice it would be most helpful in separating cyclosporin toxicity from ATN and acute rejection. The ability to discriminate ATN from Acute rejection is not sufficiently high to be useful in clinical practice.

Thus, enzymuria assay provides a simple, reliable, noninvasive method to distinguish cyclosporin nephrotoxicity from acute tubular necrosis and acute rejection in renal allograft recipients.

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