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Urinary excretion of renal brush border membrane enzymes in leprosy patients – effect of multidrug therapy

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Abstract. Renal function at the brush border membrane level has been studied using characteristic enzymes, such as alkaline phosphatase, leucine-aminopeptidase and gamma-glutamyl transpeptidase. Urinary enzyme studies were performed using leprosy patients, classified on the basis of bacteriological index (BI > 3; n = 20, BI < 3; n = 12, BI-ve; n = 10) and compared with control subjects (n = 10). The role of enzymuria in monitoring WHO-recommended multidrug therapy (MDT) has been evaluated in these patients. A significant increase in the enzyme activities ($p < 0.01$), as well as significant ($p < 0.01$) proteinurea in 24-hour urine samples of both the smear positive groups (BI > 3, BI < 3) prior to therapy compared to control subjects, indicates proximal tubular functional impairment at brush border membrane level. In the smear negative (BI-ve) group, no significant difference was observed in enzyme activities as compared with the control group. In a follow-up study (BI > 3; n = 13, BI < 3; n = 4) the activities of all the enzymes decreased significantly in all the groups when compared to a corresponding untreated group. The follow-up study was not carried out on the smear negative group. The surprising finding was the differential behaviour of r-glutamyl transpeptidase, whose activity increased significantly ($p < 0.01$) even after therapy in BI > 3 group when compared with untreated patients. However in a detailed work-up including hepatic and renal function tests, the serum biochemistry was found to be normal both before and after therapy. Urinary excretion of brush border enzymes seems to be related to bacterial load, and their potential in studying the effect of MDT remains unclear.
Key words. Alkaline phosphatase; leucine-aminopeptidase; gamma-glutamyl transpeptidase; bacteriological index; multidrug therapy; leprosy.

Leprosy, a chronic granulomatous infection, has been found to be associated with involvement of almost all organ systems of human body. Renal abnormalities such as amyloidosis and various types of nephritis observed in leprosy provide a good index of kidney involvement in the disease[1,2]. Urinalysis of immunoglobulin levels, enzymuria, antigen detection and hormonal levels could all serve as useful non-traumatic means of providing an insight into the involvement of the organ system in question[2–6]. Considerable attention has recently been paid to the determination of urinary enzymes in patients with diseases of urinary tract, and a great deal of information has been obtained concerning urinary enzyme determination[7–9]. In a preliminary study on 25 lepromatous leprosy patients, activities of the renal brush border membrane enzymes were found to be elevated significantly in comparison with those of control subjects[4]. The shedding of the tubular epithelial membrane (and consequently the enzymes) might occur before the histopathological damage and this enzymuria could be useful as an early marker of renal damage. In the present study, we have attempted to see if enzymuria could be used to monitor leprosy patients on multidrug therapy and to correlate this with other biochemical parameters.

Methods

Patients. A total of 42 patients from the leprosy clinic of the Nehru Hosptial attached to the Postgraduate Institute of Medical Education and Research, Chandigarh (India) were included in the study. These have been classified on the basis of bacteriological index (BI) i.e. BI > 3 (n = 20), BI < 3 (n = 12) and BI-ve (n = 10) and compared with control subjects (n = 10). Bacterial Index is defined as the density of bacilli in smears, including both living and dead bacilli. Ridley's logarithmic scale has been recommended for recording BI[10]. Patients of all the groups were freshly diagnosed, untreated for leprosy, had not taken any drug during the past six months, and had no history of any disease affecting the kidneys. All patients were subjected to skin biopsy and slit skin smears were collected from six sites for determination of bacteriological and morphological indices. The patients were classified according to Ridley Jopling's classification for leprosy[11]. A detailed work-up

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including haemogram, hepatic and renal function tests, serum biochemistry, urinalysis and skiagram of chest was carried out prior to MDT and at six-monthly intervals thereafter. For each patient, a 24 h urine sample collection was done in a sterilized container containing sodium azide (1 g/l). Simultaneously the clotted blood from each patient was collected in a plain vial for serum analysis. Urine samples were kept refrigerated (4 °C) after collection and were assayed in an air conditioned room. The urine samples were centrifuged at 15,000 rpm and the supernatant was dialysed against distilled water for 24 h before analysis for enzymes. Assays were performed within three months of collection.

Follow-up study. The patients were treated with a WHO multibacillary regimen consisting of rifampicin 600 mg and clofazamine 300 mg once a month given under supervision, and dapsone 100 mg daily and clofazamine 50 mg daily, self administered[12]. Follow-ups of smear positive patients only, i.e. BI > 3 and BI < 3 groups, were included in the study. Out of 32 multibacillary patients, only 17 patients (BI > 3, n = 13 and BI < 3, n = 4) returned after treatment. The follow-up study of the smear negative patients was abandoned as the number of patients returning for follow-up was too small.

Estimation of brush border membrane (BBM) enzymes. The enzymes studied were a) alkaline phosphatase[13], b) leucine-aminopeptidase[14], and c) gamma-glutamyl transpeptidase[15], using the substrates a) p-nitrophenyl phosphate in 0.5 M glycine buffer, b) L-leucine-p-nitroanilide, and c) gamma glutamyl-p-nitroaniline in Tris HCl buffer respectively. Enzyme assay for each patient was done in triplicate and the enzymatic activities were estimated spectrophotometrically (LKB-Biochrom Ultraspec 4050) as determined by the coloured products formed. Urinary protein was measured by the conventional method of Lowry et al.[16].

Results

The activities of the different renal brush border enzymes estimated before therapy are shown in table 1. A significant increase was seen in the activities of leucine aminopeptidase ($p < 0.05$) and r-glutamyl-transpeptidase ($p < 0.001$), both in BI > 3 and BI < 3 groups compared to control subjects. Similarly the activity of alkaline phosphatase was increased in both these groups, but this increase was statistically non-significant. The excretion of r-glutamyl transpeptidase was even more pronounced in both the groups compared with that of the control group. However, in the BI-ve group no significant difference in enzyme activities was observed compared to the control group. The 24 h urinary protein was found to be increased significantly ($p < 0.001$) in all three groups compared to control subjects (table 2).

Effect of treatment. The pattern of urinary excretion of the brush border enzymes showed a marked response to the multidrug regime administered to leprosy patients. In both the groups, alkaline phosphatase and leucine aminopeptidase activity decreased significantly ($p < 0.05$) after therapy compared to that in untreated patients. Conversely, r-glutamyl transpeptidase showed a significant ($p < 0.01$) rise in activity after therapy in the BI > 3 group when compared with untreated patients whereas its activity decreased significantly in BI < 3 compared to the group before therapy (table 3).

Table 1. Urinary enzymatic activities (μmol/min/mg protein) in leprosy patients and controls before drug therapy.

Group	Alkaline phosphatase (AP)	Leucine aminopeptidase (LAP)	Gamma-glutamyl transpeptidase (r-GT)
BI > 3 (n = 20)	5.27 ± 0.142	4.45 ± 1.02*	32.97 ± 16.70***
BI < 3 (n = 12)	4.76 ± 1.68	4.92 ± 1.47*	14.90 ± 0.93***
BI-ve (n = 10)	2.17 ± 0.90	2.20 ± 0.59*	1.40 ± 0.59
Controls	3.29 ± 0.68	2.27 ± 0.07	3.41 ± 0.53

*($p < 0.05$); ***($p < 0.001$) compared to control.

Table 2. Protein levels (mg/ml) in leprosy patients and controls.

Leprosy patient	Protein estimation (mg/ml) Mean ± SE
BI > 3 (n = 20)	1.01 ± 0.30***
BI < 3 (n = 12)	1.39 ± 0.49***
BI-ve (n = 10)	1.37 ± 0.52***
Controls (n = 10)	0.14 ± 0.06

***($p < 0.001$) compared to controls.

Table 3. Urinary enzymatic activities (u mole/min/mg protein) in leprosy patients before therapy (BT) and after therapy (AT).

Leprosy patients		Alkaline phosphatase (AP)	Leucine aminopeptidase (LAP)	Gamma-glutamyl transpeptidase (r-GT)
BI > 3 (n = 13)				
	BT	451 ± 1.06	3.65 ± 0.97	32.8 ± 0.02
	AT	1.99 ± 0.31	1.08 ± 0.03*	45.0 ± 21.6
BI < 3 (n = 12)				
	BT	5.38 ± 2.40	9.97 ± 3.69	31.2 ± 2.70
	AT	2.94 ± 0.80*	5.55 ± 0.02*	20.0 ± 1.14*

*($p < 0.05$) as compared to corresponding BT group.
Values have been expressed as mean ± SEM.

The urinary protein levels decreased significantly ($p < 0.05$) after therapy in comparision with the untreated levels in all three groups (table 4). Renal functions, as assessed by biochemical analysis of serum samples of patients, were found to be within normal limits in all the groups, although the levels after therapy were decreased compared to the levels before therapy (table 5), although the decrease was not statistically significant. Finally, renal functions were determined based on the enzyme levels and proteinuria.

Discussion

An understanding of the function of the renal brush border membrane can best be achieved by studying the characteristic enzymology of the proximal tubule. Necrosis of proximal tubules resulting in damaged renal basement membrane ultimately leads to shedding of the brush border enzymes, such as r-glutamyl transpeptidase, alkaline phosphatase, leucine aminopeptidase and maltase, into the urine[4]. However, in recent times, renal involvement studies in leprosy have been abandoned mainly due to the trauma of renal biopsy, insufficient biopsy material and noncompliance by relatives in providing kidneys during autopsies.

Urinary excretion of brush border enzymes serves as a useful marker in predicting renal involvement long before the development of overt clinical or pathological symptoms. Various workers have studied the characteristic renal brush border membrane enzymes as markers of tissue injury or to evaluate the protective role of agents like antipilli antibodies in pylonephritic rats[17]. The diagnostic value of r-glutamyl transpeptidase and alkaline phosphatase has been reported in serum estimations and liver scans in metastatic liver disease[18]. In addition, the levels of renal brush border membrane enzymes have been used to assess the effect of dietary cholesterol in guinea pigs[19].

In this study, we observed significant urinary excretion of all the three enzymes in both bacteriologically positive and negative untreated patients, in comparison to the control group (table 1). Proteinuria was also statistically significant but serum creatinine levels in patients of these groups were found to be in the normal range (0.6–1.2). Hence our findings suggest that enzymuria in untreated patients of all the three groups probably reflects renal dysfunction at tubular level without much effect on glomerular function. Urinary excretion of enzymes seems to be related to bacterial load as indicated by the significantly higher activities of enzymes in the BI > 3 group than in the BI-ve group.

Multidrug therapy (WHO regime) administered to all the groups was found to affect the enzymatic levels in urine samples. In both the groups of smear positive patients, the activities of urinary alkaline phosphatase, leucine aminopeptidase and urinary protein levels decreased significantly following treatment. Following treatment, r-glutamyl transpeptidase was excreted at raised levels in urine in the BI > 3 group while the levels of this enzyme decreased in the BI < 3 group. This can be explained if in the BI > 3 group, bacterial load has some effect on the substrate available for the enzymes, either by changing its conformation or by degrading it. However there is no apparent explanation for this increased enzymatic activity.

Our main finding in this study is that urinary excretion of brush border enzymes reflects renal functional status at a much earlier stage in the disease course of leprosy. Similar studies in diabetic nephropathy demonstrate urinary excretion of brush border antigen and plasma proteins in the early stages of disease[7]. Similar patterns of enzyme levels in different biological fluids have been used as markers of progressive disease, for example in bronchioalveolar lavage fluid and the serum of active

Table 4. Protein estimation (mg/ml) in urine samples of leprosy patients before (BT) and after therapy (AT).

Protein estimation (mg/ml) Mean ± SE		
	BT	AT
BI > 3 (n = 13)	0.83 ± 0.02	0.37 ± 0.14*
BI < 3 (n = 4)	1.78 ± 0.98	0.16 ± 0.07*

*($p < 0.05$) compared to corresponding before therapy group.

Table 5. Biochemical analysis of sera of smear positive patients before therapy (BT) and after therapy (AT).

Group of patients		Creatinine	Uric acid	Calcium	Phosphorous	Urea
BI > 3 (n = 13)						
	BT	0.83 ± 0.45	3.7 ± 1.93	8.47 ± 2.61	7.25 ± 5.08	22.42 ± 13.83
	AT	0.76 ± 0.26*	3.65 ± 1.58	9.13 ± 3.12	6.52 ± 4.78	25.00 ± 6.35
BI < 3 (n = 4)						
	BT	0.97 ± 0.37	3.84 ± 1.81	11.04 ± 6.68	4.51 ± 1.71	34.64 ± 20.00
	AT	0.78 ± 0.25*	3.46 ± 1.22	10.4 ± 3.44	5.43 ± 3.57	27.54 ± 8.95*

Values are expressed in mean ± SEM.
*The level of significance is *$p < 0.01$ as compared to its corresponding group before treatment.

pulmonary tuberculosis patients[8]. Similarly, an altered pattern of brush border enzymes has been reported in intestinal amoebiasis[9]. Multidrug therapy for leprosy showed a tremendous effect on the status of urinary brush border enzymes as the values tended to return to normal. However a surprising finding is that one of the enzymes, r-glutamyl transpeptidase, has been found to be excreted excessively even after therapy. Increased levels of r-GT could be due to increased synthesis of this enzyme, which could not be proven on the basis of experimental data generated from the present study. However, it is reasonable to suggest that urinary excretion of brush border enzymes could throw light on renal involvement in early states of leprosy although the influence of multidrug therapy is still questionable.

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