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Serum β-glucuronidase in subtypes of leprosy

Joseph George, M. Rajendran & V.N. Bhatia

Central Leprosy Teaching & Research Institute, Chengalpattu

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Serum β-glucuronidase activity was estimated using phenolphthalein mono-β-glucuronic acid as substrate in 176 individuals including 72 lepromatous leprosy patients, 24 patients of borderline leprosy, 42 of borderline tuberculoid and 38 healthy controls. Of these, 35 patients (20 with lepromatous leprosy, 5 with borderline leprosy and 10 with borderline tuberculoid) were untreated. The enzyme levels were increased significantly in all types of leprosy, the highest levels being seen in treated lepromatous leprosy patients (105.0 SU). There was also a significant difference in the enzyme activity between untreated patients and those on combined dapsone and rifampicin therapy, in all three types of leprosy. Among untreated patients, the maximum value observed in lepromatous leprosy was 93.4 SU. The lowest enzyme level in healthy control was 19.5 SU and the maximum was 54.0 SU. The results suggest that in leprosy patients, especially in those on daily multidrug regimens, there is an extensive damage of leucocytes and liver cells where the enzyme is largely present.

Elevated levels of β-glucuronidase have been observed in treated and untreated patients of lepromatous leprosy[1]. The enzyme levels have also been found to be increased in *Mycobacterium leprae* infected tissues of mouse and armadillos[2]. The present study aims at investigating serum levels of the lysosomal enzyme, β-glucuronidase in subtypes of leprosy and to see the effect of various antileprosy drugs on the enzyme.

Material & Methods

A total of 138 leprosy patients comprising 42 borderline-tuberculoid (BT), 24 borderline-lepromatous (BL), 72 lepromatous-lepromatous (LL) and 38 healthy controls were studied for β-glucuronidase activity. A few patients with tuberculoid-tuberculoid (TT) leprosy (who were available for the study) were included in the BT group. Similarly, a few patients of borderline-borderline (BB) leprosy were included in the BL group. None of the leprosy patients were in the reactive phase. The patient group included both out-patients as well as in-patients.

Staff members and trainees at our Institute served as healthy controls. Individuals who had regular contact with leprosy patients were excluded as controls. Only adults between the ages of 20 and 60 yr were included in the control and patient groups. Random sampling procedure was not used for selection of patients as well as healthy controls. The blood was collected by veni puncture using disposable syringe and the serum was separated after two hours incubation at 37°C.

Among the 72 lepromatous leprosy patients, 20 were active but untreated. Among the treated patients, 13 were on 100 mg dapsone monotherapy for over a period of two years, 15 received 100 mg dapsone and 600 mg rifampicin daily for about 15 days under a special study while the remaining 24 patients received dapsone (100 mg daily), rifampicin (600 mg) and clofazimine (300 mg) as pulse therapy

(pulsed MDT) over a period of one year. Out of 24 BL patients, 5 were active but untreated, 7 were on dapsone (100 mg) monotherapy for over 1-2 yr, 4 received dapsone (100 mg) and rifampicin (600 mg) daily for 15 days and the remaining 8 were given pulsed MDT for about six months. Amomg BT patients, 10 were untreated, 13 were on dapsone monotherapy, 11 were on the special study regimen (100 mg dapsone and 600 mg rifampicin daily for 15 days) and the remaining 8 were on pulsed MDT for about six months. The LL and BL patients were given pulse therapy after regular MDT for 14 days. The daily MDT regimen was not given to BT patients. The samples were collected three hours after the administration of drug in the case of pulsed MDT as well as regular rifampicin therapy.

The estimation of β-glucuronidase was carried out only in fresh sera. Haemolysed sera and those from patients with hepatitis were not included in the study. The sera from patients having other diseases which may give a rise in serum β-glucuronidase activity were also not included.

Enzyme assay : The β-glucuronidase activity was determined as per the procedure (no. 325) prescribed by Sigma Diagnostics, which is similar to the one proposed by Fishman *et al*[3]. In this procedure, β-glucuronidase acts on the substrate phenolphthalein mono-β-glucuronic acid and liberates free phenolphthalein at 56°C. The β-glucuronidase kit was procured from Sigma Diagnostics, St. Louis, MO, USA.

The assay mixture contained 0.6 ml acetate buffer solution, 0.2 ml substrate (phenolphthalein mono-β-glucuronic acid, 0.03 mol/1, *p*H 4.5 at 25°C) and 0.2 ml serum. The tubes were incubated for one hour at 56°C in a water bath. Immediately after incubation, 5ml AMP (2-amino-2-methyl-1-propanol) buffer was added. The milky precipitate formed by addition of serum disappeared with mixing of AMP buffer. The intensity of the resultant red colour was measured in a Varian double beam spectrophotometer (model 634S) with a 10 mm cuvet at 550 nm. The activity of the enzyme was expressed in Sigma Units. One modified Sigma Unit of β-glucuronidase liberates 1 μg of phenolphthalein from phenolphthalein glucuronic acid per hour at 56°C.

Statistical analysis was done using Student's 't' test. In LL type, compared to healthy controls, logarithmic transformation was used to stabilize the variance.

Results

The enzyme activity was calculated from a calibration curve made by plotting optical density (at 550 nm) versus phenolphthalein concentrations (μg/ml). The samples which were out of range of the calibration curve were calculated from the regression equation of the calibration curve.

The β-glucuronidase levels were greatly increased in untreated lepromatous leprosy patients compared to healthy controls (Table I). There was a gradual increase in the enzyme level from BT to LL type. Compared to healthy controls, the enzyme level in LL as well as BL types were statistically highly significant ($P < 0.001$). The values for healthy control versus BT and BT versus LL were

Table I. Serum β-glucuronidase activity (mean±SE) in untreated leprosy patients (modified Sigma units/ml)

Type of leprosy	β-glucuronidase activity	Range	Level of significance		
			HL vs BT, BL & LL	BT vs BL & LL	BL vs LL
Healthy controls (HL)	37.42±1.41 (n = 38)	19.50 – 54.00	–	–	–
Borderline tuberculoid (BT)	43.32±2.07 (n = 10)	33.00 – 60.50	$0.025 < P < 0.05$	–	–
Borderline lepromatous (BL)	50.80 ± 4.60 (n = 5)	39.50 – 62.00	$P < 0.001$	NS	–
Lepromatous-lepromatous (LL)	57.98 ± 2.72 (n = 20)	44.00 – 93.50	$P < 0.001$	$0.001 < P < 0.005$	NS

NS, not significant.

Table II. Serum β-glucuronidase activity according to treatment (modified Sigma units/ml) (Data are mean ± SE)

Type of treatment	BT patients (n = 42)**		BL patients (n = 24)		LL patients (n = 72)	
	Enzyme activity	*P*-value (in comparison with untreated)	Enzyme activity	*P*-value (in comparison with untreated)	Enzyme activity	*P*-value (in comparison with untreated)
Untreated	43.32±2.07 (n=10)	–	50.80±4.60 (n=5)	–	57.98±2.72 (n=20)	–
DDS 100 mg	44.96±2.13 (n=13)	NS	51.00±4.70 (n=7)	NS	65.82±2.76 (n=13)	NS
DDS 100 mg + rifampicin 600 mg	56.40±2.51 (n=11)	$P < 0.01$	62.07±4.62 (n=4)	NS	74.58±4.42 (n=15)	$P < 0.01$
DDS 100 mg + rifampicin 600 mg* + clofazimine 300 mg*	45.25±3.02 (n=8)	NS	58.46±3.64 (n=8)	NS	69.96±3.26 (n=24)	$0.01 < P < 0.02$

* only pulse therapy.
** Clofazimine is not given under pulse therapy.
NS, not significant

also statistically significant but the values of BT versus BL and BL versus LL were not significant.

In LL and BT types of leprosy, there was a significant difference in the enzyme activity between untreated patients and those on combined daily dapsone and rifampicin therapy, but in BL type the difference was not significant (Table II). This may be due to very low number of patients (four) available in that particular group. There was no significant difference in the enzyme activity between untreated patients and those on regular dapsone monotherapy in all types of leprosy. In multidrug regimens where rifampicin and clofazimine has been received as a pulse therapy, there was also a significant increase in the enzyme activity in LL patients, but the difference was not significant in BL and BT patients.

There were no significant differences in the enzyme levels between the two sexes in all the three types of leprosy patients (Table III).

Discussion

The lysosomal enzyme β-glucuronidase is abundant in liver, pancreas and other tissue[4]. Increased circulating concentrations of the enzyme may be of clinical significance in several diseases including different types of leprosy. Palekar and Magar[5] reported increased activity of lysosomal enzymes *viz.*, acid phosphatase, cathepsin, ribonuclease and aryl sulphatase in leprosy tissues. Suzuki and associates[6] observed significantly high lysozyme activity in lepromatous leprosy patients with erythema nodo-

Table III. Serum β-glucuronidase activity (mean ± SE) in male and female (modified Sigma Units/ml)

Type of leprosy	β-glucuronidase activity	
	Male	Female
Healthy controls (HL)	38.67±1.96 (n = 21)	35.88±2.02 (n = 17)
Borderline-tuberculoid (BT)	48.91±1.71 (n = 24)	46.01±2.43 (= 18)
Borderline-lepromatous (BL)	57.42±2.60 (n = 15)	55.4±4.31 (n = 9)
Lepromatous-lepromatous (LL)	67.20±2.35 (n = 46)	67.80±3.00 (n = 26)

The differences between male and female patients were not significant.

sum leprosum (ENL) reaction. They also observed an increased lysozyme activity in BT patients. According to Dastur and co-workers[1], both treated and untreated leprosy patients showed increased lysosomal enzyme activity evidenced by single or paired paranodal spots of acid phosphatase and β-glucuronidase in Schwann cells in histochemical preparations of the nerve.

Venkatesan and co-workers[7] noticed remarkable increase in β-glucuronidase levels in patients of lepromatous leprosy. They found maximum enzyme levels in LL patients with ENL reaction. However, Gonzalez and associates[8] observed only a slightly increased levels of β-glucuronidase, acid and alkaline phosphatases in peripheral blood leucocytes from active LL patients when compared to healthy controls. Matsuo *et al*[9] observed the presence of β-glucuronidase and lysozyme in leprous skin lesions in tuberculoid, borderline and lepromatous types. It has been reported[2] that in mouse foot-pads and in the armadillo tissues, *M. leprae* infection resulted in remarkable elevations of β-glucuronidase levels.

It is also important to note that many drugs will influence serum β-glucuronidase levels. Rifampicin is a well known and the most potent antileprosy drug[10,11]. It was reported that lysosomal activities and serum β-glucuronidase levels were increased after rifampicin chemotherapy[6,12]. In the present investigation there was a significant increase in the enzyme activity in patients on combined regular treatment with dapsone and rifampicin in LL and BT types of leprosy. Increased enzyme activity was also noticed in multi-drug regimens where rifampicin and clofazimine has been used as a pulse therapy. Rifampicin is known for its hepatotoxicity during monotherapy[13] as well as in combination with other drugs[14]. The increased enzyme level during rifampicin treatment may be due to the destruction of liver cells and simultaneous spillage of the enzyme to the blood.

Decrease in lysosomal activities after treatment with steroids and thalidomide has also been observed[6]. These drugs are mainly used in treating patients with lepra reaction. It is reported that corticosteroids may increase the stability of lysosomal membrane[15]. After treating lepra reaction with corticosteroids, a 50 per cent fall in the β-glucuronidase level was noticed when the reaction subsided[7].

Lombardo and co-workers[16] observed that β-glucuronidase levels in human plasma will be influnced by age and sex. In the present investigation only adults between the age group of 20-60 were included. The difference between the enzyme levels in male and female patients was not statistically significant. It has been reported that during pregnancy β-glucuronidase levels will be increased[17]. In the present study, however, pregnant women were not included.

Our study also showed a marked increase in β-glucuronidase levels in LL patients when compared to healthy controls, corroborating the earlier reports. The maximum enzyme activity encountered in the present study was 93.5 Sigma Units per ml serum in untreated lepromatous leprosy while the lowest was 44. The increased level of β-glucuronidase in leprosy patients, especially in LL patients may be due to the high level of the destruction of leucocytes where lysosomes and β-glucuronidase are largely present.

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Reprint requests: Dr. V.N. Bhatia, Director (Microbiology), Central Leprosy Teaching and Research Institute, Chengalpattu 603001