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Serum beta-glucuronidase levels in children with leprosy

DEVKI NANDAN*, K. VENKATESAN**,
KIRAN KATOCH** & R.S. DAYAL***
**RML Hospital, New Delhi 110 002, India*
***National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Taj Ganj, Agra 282 001, India*
****S.N. Medical College, Agra 282 002, India*

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Summary A study was undertaken to find out the usefulness of determining the circulating levels of beta-glucuronidase, a lysosomal enzyme in leprosy affected children of less than 15 years of age. The serum enzyme levels were significantly higher in BB/BL patients compared to healthy control children as well as children with skin diseases other than leprosy. Treatment with Multidrug regimen advocated by WHO for multi/paucibacillary leprosy resulted in a significant fall in the serum enzyme levels in BB and BL cases. The findings suggest that serum beta-glucuronidase may be a useful parameter for the activity and extent of pathogenesis in leprosy.

Introduction

Leprosy is considered to be a special public health problem, owing to the permanent disabilities it causes as well as its social consequences such as discrimination and stigma. At the beginning of 2005, the global registered prevalence of leprosy was 286,063 cases and the number of new cases detected during 2004 was 407,791.[1] Among them, 47% were multibacillary, 13·2% were children, and 4% were diagnosed with severe disabilities. A very high prevalence rate of 33·9/10,000 has been reported in urban Agra and the prevalence among children was 4·4/10,000.[2] Studies on leprosy patients attending the urban leprosy centre/clinic have reported incidence rates of 5–10% for children in the age group of 0 to 14 years among leprosy patients.[3,4] There are reports on the blood and tissue levels of lysosomal enzymes, viz. lysozyme, beta-glucuronidase and N-acetyl beta-glucosaminidase in adult leprosy correlating the levels with activity of the disease.[5–10] Since leprosy has been identified as an important health problem in children, this study was conducted in children to

Correspondence to: K. Venkatesan, National JALMA Institute for Leprosy, Taj Ganj 282 001, India (Fax: +91 562 233 1755; e-mail: venkatesan_52@rediffmail.com; venkatesank@icmr.org.in)

evaluate the levels of beta-glucuronidase as a marker of activity and pathogenesis of the disease.

Materials and methods

Eighty-two children of up to 15 years of age were included in this study. The age-wise and sex-wise distribution of the subjects is presented in Table 1. The children with leprosy (51) were those who attended the Outpatient Department of the National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra (India), and they were clinically categorised using defined criteria.[11] Nineteen children with leprosy who had not taken leprosy treatment prior to the study were re-examined after 1 month of treatment (dose adjusted according to age) with either paucibacillary MDT of rifampicin once monthly and dapsone daily (TT and BT cases), or multibacillary MDT of monthly rifampicin along with daily dapsone and clofazimine (BB and BL cases). Ten children having non-leprotic skin diseases (psoriasis/atopic dermatitis/contact dermatitis) and 21 healthy children were included in this study as controls.

Venous blood was drawn from all subjects and the sera were separated and stored at $-20\,^{\circ}C$. Enzyme beta-glucuronidase levels were determined spectrophotometrically by the method of Fishman using phenolphthalein monoglucuronide as substrate.[12] One unit of enzyme activity was equal to the amount of enzyme which liberated 1 ug of phenolphthalein from a 1 mM solution of phenolphthalein monoglucuronide in 1 hour at pH 4·5 at $38\,^{\circ}C$. The results were expressed as Mean $\pm$ S.E. Statistical significance of the findings between healthy children/children with non-leprotic skin diseases and those with leprosy as also the significance between treated and untreated groups was evaluated using students' 't' test.

Results

The levels of serum BG in children with leprosy as well as non-leprotic skin diseases are presented in Table 2. The BG activity was increased in all subtypes of leprosy except in TT and indeterminate leprosy (data not shown) compared to healthy controls. But the children

Table 1. Profile of patients and controls

	Male		Female	
Subjects	<5 years	6–15 years	<5 years	6–15 years
Healthy controls	4	8	5	4
Indeterminate leprosy	–	–	–	2
Tuberculoid Leprosy (TT)	–	9	–	–
Borderline Tuberculoid (BT)	4	10	–	5
BB Leprosy	1	10	–	–
BL Leprosy	–	8	–	2*
Other skin diseases (psoriasis 4, contact dermatitis 3, atopic dermatitis 3)	–	6	–	4

* Of the two children with BL, one had ENL prior to this study and was in the subsidence phase consequent to treatment with steroids.

Table 2. Serum beta-glucuronidase activity in children with leprosy

Subjects	Enzyme activity (Units/dl) ± SE
Healthy Controls (21)	151 ± 8·91
Non-leprotic skin disease (10)	213 ± 10·47
Children with TT leprosy (09)	143 ± 10·60
Children with BT leprosy (19)	209 ± 18·90*
Children with BB leprosy (11)	339 ± 38·40*$
Children with BL leprosy (10)	353 ± 54·28*$

*$P < 0·001$ versus Healthy controls; $$P < 0·001$ versus Non-leprosy skin diseases.

wit BL and BB leprosy only had increased serum enzyme levels compared to the children with skin diseases other than leprosy. One BL case who had reaction prior to this study and was in subsidence phase of the reaction at the time of this study still had high serum enzyme levels.

The data on the effect of treatment on serum BG activity in children with sub-types of leprosy (according to the Ridley-Jopling classification) are presented in Table 3. The enzyme activity that was found decreased significantly in BB and BL cases only subsequent to treatment.

Discussion

Palekar and Magar[10] reported increased activity of four lysosomal enzymes viz. acid phosphatase, cathepsin, ribonuclease and aryl sulphatase in leprosy tissues. The lysosomal enzyme beta-glucuronidase is abundant in leucocytes and liver and hence increased circulating concentration of the enzyme may be of clinical significance in several diseases including leprosy. George *et al.*[8] have reported increased serum BG activity in all types of leprosy with the highest level in untreated lepromatous leprosy patients. Vaishnavi *et al.*[9] have also observed increase in specific activity of serum hydrolytic enzymes-alkaline phosphatase, N-acetyl beta-glucusaminidase and beta-glucuronidase in all types of leprosy patients and suggested that the increase in circulating hydrolytic enzymes could be a tissue damaging factor and may be responsible for many of the lesions seen in leprosy. Our earlier study has shown increased serum BG levels in active adult lepromatous leprosy patients and

Table 3. Serum BG activity in leprosy children before and after treatment

	BG activity (Units/dl) (Mean ± SE)	
Subjects	Before treatment	After treatment
Children with TT (*n*-4)	174·11 ± 20·51	171·25 ± 20·10
Children with BT ($n = 5$)	187·25 ± 23·23	179·38 ± 22·14
Children with BB ($n = 6$)	350·34 ± 34·24	168·28* ± 42·35
Children with BL ($n = 5$)	362·30 ± 36·25	258·50* ± 45·40

*$P < 0·001$

LL patients with ENL or in subsiding phase of the reaction.[6] The activity of two lysosomal enzymes - aryl sulphatase and cathepsin D - has been reported to be higher in the sera of patients (age ranging between 15 and 61) with psoriasis suggesting some role of these lysosomal enzymes in the pathogenesis of psoriasis.[13] In the present study also, although the enzyme levels were found significantly raised in all subtypes of leprosy compared to healthy controls, the increase was significant in BB and BL only compared to other control groups with non-leprosy skin diseases, suggesting a role of the enzyme in the pathogenesis of leprosy.

A significant fall in serum enzyme level was found only in BB and BL cases with Multidrug therapy in the present study. Palekar and Magar[10] have reported that specific activities of lysosomal enzymes from tissues of leprosy patients of all types decreased significantly with a tendency to reach normal values in multibacillary patients after treatment with DDS. On the contrary, George *et al.*[8] have shown an increase in serum BG levels in treated LL patients compared to untreated patients and suggested an excessive damage of leucocytes and liver cells where the enzyme is largely present as the cause for increased enzyme level in serum.

Conclusion

The findings of the present study suggest that serum beta-glucuronidase may be a useful parameter for the activity and extent of pathogenesis in leprosy.

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