SKIN TESTS FOR EVALUATION OF CELL MEDIATED IMMUNITY IN LEPROSY

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Cell-mediated immune (CMI) response to lepromin and dinitrochlorobenzene (DNCB) was evaluated in 60 freshly detected leprosy cases. It was observed that 70% (28 of 40) of the patients across the leprosy spectrum except LL cases revealed delayed hypersensitivity to DNCB as against 42.5% (17 of 40) to lepromin. DNCB test was found superior to lepromin test to measure CMI because of its simplicity and easy interpretation of skin reactivity. It detected CMI in 40% of BL cases who were lepromin negative. Grading of skin reactivity showed a progressive decrease in delayed hypersensitivity across the spectrum of leprosy from TT to LL. It can be concluded that there is no gross impairment of non-specific CMI in leprosy patients other than LL cases and this non-specific CMI depression correlates well with Ridley-Jopling clinical scale of leprosy.

Key words : CMI, Lepromin, DNCB, Leprosy.

Rees et al[1] showed that a disease similar to lepromatous leprosy could be produced in experimental animals after total depression of cell-mediated immunity (CMI) by thymectomy and deep X-irradiation. This observation created a great interest among several workers[2-5] to assess the role of CMI across the leprosy spectrum. A battery of in vivo and in vitro tests have been developed for evaluation of CMI. Epidemiological studies have revealed that persistently lepromin negative individuals have a high risk of lepromatous leprosy.[6] Some studies have shown that all leprosy patients have a generalised impairment of CMI when compared with healthy persons.[4]

The present study was undertaken to assess the CMI state in leprosy patients across the spectrum of the disease using specific lepromin test and the non-specific DNCB test. It was also attempted to measure any minor differences of CMI level within the leprosy spectrum by quantitating skin reactivity.

Materials and Methods

Sixty leprosy patients of both sexes and 10 healthy adults as controls formed the subjects of this study. Only such cases who were definite and classifiable unequivocally according to Ridley-Jopling clinical scale[7] were included in the study and comprised of 10 TT (Tuberculoid), 10 BT (Borderline tuberculoid), 10 BB (Borderline), 10 BL (Borderline lepromatous) and 20 LL (Lepromatous) types of leprosy.

Lepromin test and DNCB test were performed as detailed below and skin reactivity in leprosy patients and control persons was measured and the responses were graded into 4 categories ranging from 0 to 3+.

(a) Lepromin test : 0.1 ml of Dharmendra antigen prepared at the Central JALMA Institute, Agra, was injected intradermally into the flexure surface of right forearm. The early reaction was measured at 24 and 48 hours after the inoculation and an induration of 5 mm and above with erythema of 10 mm or more was considered as positive. The responses were graded as 0 (less than 5 mm), 1 + (5-9 mm), 2 + (10-14 mm) and 3 + (15 mm and above). The late reaction was recorded at 3 and 4 weeks by measuring the induration only, biopsy was not done although it is essential for the late reaction.

(b) DNCB test : Analytical grade of 1-chloro, 2, 4 dinitrobenzene was used and the method of Catalona et al[8] was employed. Volar aspects

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of the left forearm and upper arm were cleaned with acetone, and after drying the site 1000 μg (0.1 ml of 1%) and 100 μg (0.1 ml of 0.1%) of DNCB in acetone was delivered drop by drop from a tuberculin syringe on the prepared skin enclosed by a steel ring of 2 cm diameter. The area thus sensitised was covered with the bandage for 24 hours. The test site was examined at 24 hours for any irritant reaction and at 7 and 14 days. If no spontaneous flare occurred within 2 weeks, a challenge dose of 10 μg (0.1 ml of 0.01%) was applied on the flexure surface of the left forearm in the immediate proximity of the original application i.e. sensitised site. Skin reaction to DNCB was classified into 4 categories : Spontaneous flare with erythematous induration and itching within 2 weeks (3 +), spontaneous flare only (2+), delayed hypersensitivity after challenge (1 +) and no reaction after challenge (0).

Results

DNCB test revealed irritant skin reaction 1-2 days after the application in 6 control subjects and 14 leprosy patients which subsided within 4 days. Table I shows the responses to DNCB tests in different types of leprosy cases and controls. DNCB skin reactivity was seen in 9 (90%) of 10 controls and 28 (47%) of 60 leprosy patients. Among leprosy cases, the skin response to DNCB was 90% in TT, 80% in BT, 70% in BB, 40% in BL and nil in LL types. Quantitative grading of DNCB sensitisation revealed progressively decreasing values across the leprosy spectrum from TT to LL.

The responses to lepromin test in leprosy patients and controls are given in table II. Lepromin test was positive in 7 (70%) of 10 controls and 17 (28%) of 60 patients. Among the leprosy patients, skin reactivity to lepromin

Table I. Skin reaction to DNCB test in leprosy patients and controls.

Type of cases	Tested	Number of cases With grade of skin reaction				Total number (%) positive
		—	+	++	+++	
Controls	10	1	3	4	2	9 (90)
Tuberculoid	10	1	2	4	3	9 (90)
Borderline tuberculoid	10	2	3	3	2	8 (80)
Borderline	10	3	4	3	0	7 (70)
Borderline lepromatous	10	6	3	1	0	4 (40)
Lepromatous	20	20	0	0	0	0

Table II. Skin responses to lepromin test in leprosy patients and controls.

Type of cases	Tested	Number of cases With grade of skin reactivity as the induration in mm				Total number (%) positive
		—	+	++	+++	
Controls	10	3	3	3	1	7 (70)
Tuberculoid	10	3	2	3	2	7 (70)
Borderline tuberculoid	10	4	5	1	0	6 (60)
Borderline	10	6	4	0	0	4 (40)
Borderline lepromatous	10	10	0	0	0	0
Lepromatous	20	20	0	0	0	0

was 70% in TT, 60% in BT, 40% in BB and nil in BL and LL types. The intensity of cutaneous response was progressively decreasing along the spectrum of the disease from TT to BB-LL.

Comments

The results of our study indicate that DNCB test, besides revealing sensitisation of 90%, 80% and 70%, as against lepromin reaction of 70%, 60% and 40% in TT, BT and BB cases respectively, also revealed skin reactivity in 40% of BL cases who were lepromin negative.

As a measure of CMI, skin tests using recall antigens such as lepromin, PPD, candida antigen, mumps antigen and streptokinase-streptodornase as well as epicutaneous sensitisation to DNCB and picryl chloride have been commonly employed.

Contact sensitisation to DNCB offers several advantages over the intradermal tests in gauging the CMI status because of its simplicity, freedom from side effects and antibody production, and easy interpretation of the results. Both sensitisation and challenge can be controlled. Our findings have revealed DNCB skin reactivity in 90% of the controls and TT cases with total absence in the LL patients, thus indicating the normal prevalence of CMI in the tuberculoid type as against absolute impairment of CMI in the lepromatous type of the disease.

Rea[5] and Sivamani et al[9] have shown normal CMI (98%) in leprosy patients, while Bullock[2] and Turks and Waters[10] have found gross generalised impairment of DNCB delayed hypersensitivity in leprosy. Jayasingh and Bhatia[11] have shown DNCB sensitivity in 75 of 76 lepromatous cases; however, all our 20 LL patients showed total impairment of the DNCB sensitivity.

Further more, quantitative grading of DNCB skin reactivity has shown a gradual decrease in delayed hypersensitivity across the spectrum of leprosy from TT to LL. This indicates that non-specific depression of CMI almost correlates with Ridley-Jopling clinical scale of leprosy.

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